

This preprint by van Steenbergen *et al.* presents a laboratory behavioural study of females of a pair of closely related species of Lake Tanganyika cichlid fishes. Female *Ophthalmotilapia nasuta* showed very different responses to conspecific males (and females) in comparison to males of the closely-related *O. ventralis*. By contrast, *Ophthalmotilapia ventralis* female showed similar responses to conspecifics and heterospecifics. This was consistent with a previous study (Nevado *et al.* 2011) of wild-caught specimens that showed that *O. nasuta* populations included occasional individuals bearing mitochondrial DNA (mtDNA) sequences more characteristic of related species, including *O. ventralis*. As mtDNA is normally maternally inherited, this suggests that male *O. nasuta* occasionally mate with female *O. ventralis*. However, the scarcity of characteristic *O. nasuta* mtDNA in populations of related species suggests that female *O. nasuta* show strong preferences for conspecific males, which was consistent with van Steenbergen *et al.*'s laboratory trials. The emphasis on female choice in this study system is likely to be justified, as the cryptic-coloured female *Ophthalmotilapia* brood their eggs and young in their mouths for several days, while the flamboyant males provide no parental care, but compete to attract females. With females taking several weeks or months to complete a reproductive cycle and be ready to produce another clutch, it is likely that there is a strongly male-biased operational sex ratio. The divergence in patterns of female mating preferences is likely to help explain patterns of hybridisation seen among groups of closely related cichlid fish species, which in turn may influence the detailed course of events during adaptive radiations. This is likely to be of wider significance, given the increasing evidence of introgression even among well-differentiated non-sister lineages during adaptive radiations of cichlid fishes and many other taxa. Thus, the study has considerable significance and has results that are likely to advance our understanding of the role of behaviour in adaptive radiation and speciation.

Overall, the experiments seem to be well-designed and analysed, the results presented clearly and succinctly and the interpretation seems generally robust. Occasionally one or two things might be clearer and a couple of statements seemed to be a little too definite in relation to the evidence presented.

Lines 79-80: 'Given the importance of the initial contact, we may expect that the early response to conspecific and heterospecific mates will predict the outcome of the mating process to a substantial degree.' Perhaps this might be expressed a little more clearly in terms of the justification of the experimental design employed. The suggestion seems to be the initial behavioural response by females to conspecific and heterospecific potential mates is proposed to be a good predictor of actual mate preferences. It could be made clear whether this is being assumed or tested. Maybe we could be given a bit more background to explain why this might be a worthwhile thing to do, for example by pointing out the difficulties with other methods reported in the literature. This section might be most appropriate at the end of the introduction (lines 166-167), as it seems out of place where it is currently.

Lines 229-238 (also lines 553-557): the idea that focal 'females' had changed sex seems to be concluded rather too strongly. The evidence for sex change in cichlids is fairly weak (see lines 553-557) and taxonomically narrow, and there is nothing specifically on *Ophthalmotilapia*. The alternative explanation is that sex determination by examination of the genital papilla is not straightforward. Perhaps this ought to be mentioned as an alternative. Solid evidence for sex change would really be when known (isolated or tagged) individuals were seen to spawn (as females) and then post-sex change were found to be able to fertilise eggs of females successfully and when dissected, had male gonads with sperm visible on microscopic examination. The present study falls well short of this level of evidence: immature males and females basically look the same in most cichlids. The illustrations on Supplement 2 show that 'apparent females' that were euthanised after

developing male phenotypes (colourful) had male gonads and those that retained female phenotypes had female gonads, but this is also consistent with some of the apparent females being late-developing males.

Lines 281-285: "Each parameter was calculated three times: once using coordinates obtained for 721/871 seconds before (before) the removal of the visual barrier, once using coordinates obtained during 721/871 seconds (after1) after the visual barrier was removed and finally using coordinates obtained during 2186/2685 seconds (after2)". Does 721/871 mean that a period ranging from 721 to 871 seconds was analysed, depending on how much suitable footage had been filmed? Or that sometimes it was 721 seconds and other times it was 871 seconds (as these were the minimum 'before' frame counts in the two experiments). It might be clearer to just give a little bit of an explanation here.

Line 315: Permanova is method that may not be familiar to all readers, although it does seem appropriate and useful. It might help to briefly drop a few hints about it. For example, it might be useful to point out that Permanova is a non-parametric technique, because otherwise the use of non-parametric MW tests on the same dataset seems a bit odd. Overall, it might have been interesting to see a bit more of the data distribution shapes to get some idea of whether it was really non-parametric and why no attempt could be made to transform the data for parametric analyses, most of which are generally considered quite robust to non-normality and more statistically powerful.

Lines 459-460: "Although we could not exclude that these differences stem from dispersion effects". It might be clearer to say what is meant by 'dispersion', since it is used in a discussion about significant differences in average values. This also crops up in Table 2, and seems to reflect an analysis analogous to a test of homogeneity of variances as would normally be used in parametric tests. This would usually invalidate the analysis in parametric statistics, but it is not treated this way here. Maybe this could be briefly explained in the methods section.

Lines 647-649: "Males therefore evolved morphological characteristics, build conspicuous bowers and/or perform stereotyped displays to distinguish them from sympatric congeners." This seems an overly strong statement to the effect that species differences in courtship signals are the result of selection to reduce interbreeding (or at least time wasting courtship) between closely-related species. In theory, it could also be driven by purely intraspecific processes. The relative importance of these two factors can really only be estimated empirically and not determined a priori. Maybe make a slightly less definite statement here.