

Directional sexual selection on chroma and within-pattern colour contrast in *Labeotropheus fuelleborni*

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Speciation via intersexual selection on male nuptial colour pattern is thought to have been a major force in promoting the explosive speciation of African haplochromine cichlids, yet there is very little direct empirical evidence of directional preferences within populations. In this study, we used objective spectrophotometry and analyses based on visual physiology to determine whether females of the Katala population of *Labeotropheus fuelleborni*, a Lake Malawi haplochromine, prefer males that have higher chroma and more within-pattern colour contrast. In paired male preference tests, female Katala *L. fuelleborni* showed increasing preferences for males with more relatively saturated colours on their flanks. They also showed increasing preferences for males with relatively higher contrast levels among flank elements. This is the first empirical evidence, to our knowledge, for male colour as a directionally sexually selected trait within a haplochromine cichlid population.

Keywords: cichlid; haplochromine; sexual selection; speciation; chroma; colour contrast

1. INTRODUCTION

Female mate choice on male nuptial coloration is thought to promote speciation in the African haplochromine cichlid flocks (Dominey 1984; Stauffer *et al.* 1995). Theoretically, directional sexual selection via female choice on some aspect of male coloration may act as a reproductive isolation barrier among (or possibly within) populations of differently coloured males (Turner & Burrows 1995; Seehausen 2000). Although this phenomenon has been investigated in other taxa (Endler & Houde 1995; Boughman 2001) and preferences among haplochromine colour morphs and populations have been documented (Seehausen 2000), the role of sexual selection in the evolution of male coloration within a haplochromine population is little studied (Jordan *et al.* 2003).

Studies examining male coloration and sexual selection in general have discovered female preferences for several aspects of male colour pattern. These include the effects of the gross colour pattern, as well as fine-scale aspects of

coloration such as brightness, chroma, hue and contrast (Brooks & Endler 2001; Pearn *et al.* 2001). The effects of differences between opponent males in these measures have also been found to be important in the strength of preferences (Kodric-Brown & Johnson 2002).

In any such study, it is important to account for the perceptual abilities of the taxa in question (Endler 1990). The mbuna, or rock-dwelling haplochromines from Lake Malawi, were recently studied in this regard by Carleton *et al.* (2000), who discovered the four photoreceptors in *Metriaclima zebra* Boulenger (Stauffer *et al.* 1997). Furthermore, gene expression studies have shown that *Labeotropheus fuelleborni* shares these same photoreceptors with *M. zebra* (Carleton & Kocher 2001).

Labeotropheus fuelleborni is a shallow-dwelling mbuna from Lake Malawi (Ribbink *et al.* 1983). This species exists as a group of isolated populations distributed around Lake Malawi, with the males of each population bearing a distinct nuptial colour pattern (Ribbink *et al.* 1983). Although further details of *L. fuelleborni* colour vision would be useful, current knowledge allows for a first approximation of colour perception in this species (Endler 1990, 1991). Therefore, it provides an opportunity to examine the role of male coloration in sexual selection and speciation/population isolation.

We examined mate choice based on coloration in the Katala population of *L. fuelleborni*. Specifically, we were interested in two aspects of coloration: colour intensity (or chroma) and within-pattern colour contrast. It has been shown in other fishes that females prefer males that have both very intense colours and strongly contrasting colour pattern elements (Baube *et al.* 1995; Brooks & Endler 2001). We therefore wanted to determine whether females preferred males with higher chroma, higher colour contrast, both, or neither aspect of coloration.

2. METHODS

The Katala population of *L. fuelleborni* was used in this experiment. The Katala males have a flank that is orange on the ventral portion with a sky-blue dorsum, and a blue-white dorsal fin with a yellow trailing edge. The Katala females are grey or brownish-grey, sometimes with yellowish colour on the dorsal fin. All fish used in this experiment were wild caught.

The behavioural arena was a 300 l aquarium divided into three sections: two side compartments of *ca.* 30.5 cm long and a middle section of *ca.* 61 cm in length. The sections were separated by permanent, clear, ultraviolet (UV)-transparent Plexiglas dividers. A ceiling light fixture above the arena was fitted with one 40 W cool white fluorescent bulb and one 40 W 5.0 UVB Iguana Light (ZooMed) fluorescent bulb.

Fifteen virgin females were selected for use in the mate-choice trials. To ensure the display of courtship behaviours, a female was used in an experiment only when she was gravid. A female in such condition was placed in the centre section of the arena, and opaque plastic dividers were then placed in front of the Plexiglas dividers. Each female was used only once in this experiment. Two randomly selected males were then placed in the side compartments of the arena, one per compartment. Some males ($n=6$) were used twice during this experiment, but never paired with the same opponent (Kodric-Brown & Johnson 2002). The fishes were then allowed to acclimate for a period of between 4 and 20 h before the observation.

When the observation began, the opaque dividers were removed, and the trial was videotaped; no humans were present during the trials. After 45 min, videotaping was stopped and the fish were removed from the arena for spectrophotometric analysis. The videotape was later viewed and the number of female receptive responses to each male was counted. Trials in which females displayed aggressive behaviour or were non-responsive to courtship were discarded from the analysis.

At the conclusion of the behavioural trials, all three fish were removed from the arena and placed in a weak solution of MS 222 anaesthetic. We removed a fish from the solution when its respiration slowed and it lost its balance, then transferred it to an ice bath. The

ice bath served two important functions. First, it acted as an additional anaesthetic, so that less MS 222 was used. Second, the cold temperature of the ice bath contracted the melanophores in the shallow layers of the fish's skin, allowing the pigments that the fish would normally display during courtship to be detected by the spectrophotometer (I. Kornfield, personal communication). Each fish was left in the ice bath for *ca.* 3 min, at which time its respiration became almost undetectable and it was incapable of movement.

After removal from the ice bath, the fish was placed on a piece of black cloth underneath a UV-transmitting quartz lens mounted on a macro tube, which directed light via a fibre-optic cable into an Oriel Instaspec IV charge-coupled device attached to a FICS (Fixed Image Compact Spectrograph). The fish was illuminated from its dorsal surface by four 50 W Solux halogen lamps and a 20 W UV blacklight, providing enough illumination for reliable reflectance measurements between 350 and 725 nm. The Solux and UV bulbs were located at a distance of 80 cm away from the fish, and a height of 45 cm above the fish. Regular room lights were also on during spectrophotometry.

Colour data were recorded from eight spots on each individual fish (see figure 3 in electronic Appendix A), and were converted to actual reflectance measurements by dividing them by analogous measurements taken from a Spectralon white standard. After all reflectance measurements were made, a photograph of the fish's left side was taken. The areas of the five flank elements (operculum, forehead, upper flank, peduncle and lower flank) were measured from these photographs. Fin areas were not measured owing to inconsistencies in how the fins were pinned for photography. Fish were then placed in warm well-aerated recovery aquaria for a period of 24 h before being returned to the holding tanks.

We used the cone sensitivities of *M. zebra* as a first approximation of those of *L. fuelleborni* in calculations of both chromas and the contrast between pairs of patches on the body (see electronic Appendix A). We used two distinct models for calculating both chroma and contrast. One chroma model was based on Euclidean distances among adjacent cone pair interactions (Endler 1990), whereas the other was based on Endler's D_{mx} approach (Endler 1991). Similarly, one of our contrast models was based upon Euclidean distances between cone pair chroma for the two patches (Endler 1990), whereas the other took an approach used by Rush *et al.* (2003) involving comparisons between relative stimulation of individual cones (see electronic Appendix A).

The differences between opponent males in chroma and contrast were then regressed against the difference between the numbers of receptive responses that each received from the female. For the regression analyses, the differences between males were calculated as winner minus loser for each parameter.

3. RESULTS

Females demonstrated increasing discrimination between males as differences in male coloration increased. Regression analyses based on Euclidean distances among pairwise cone pair interactions demonstrate that a difference in the chroma of the five size-adjusted flank elements between opponents is significantly correlated with the difference in female receptive responses between opponents ($p = 0.030$; adjusted $R^2 = 0.359$; figure 1*a*). Females also displayed preferences for males that had greater colour patch contrast among these same flank elements than their opponents. Figure 1*b* shows the effect of a composite Euclidean contrast for the five size-adjusted flank elements on female response; males with greater flank element contrast than their opponents gained more responses than their opponents ($p = 0.036$; adj. $R^2 = 0.335$). Our results using the other methodologies are very similar ($p = 0.031$ for chroma as D_{mx} ; $p = 0.052$ for contrast based on relative stimulations).

The two different perceptual models employed to measure relative conspicuousness, based on winner–loser differences, were strongly correlated. Both chroma measures were significantly correlated ($p = 0.000$; adj. $R^2 = 0.844$), as were the two contrast measures ($p = 0.000$; adj.

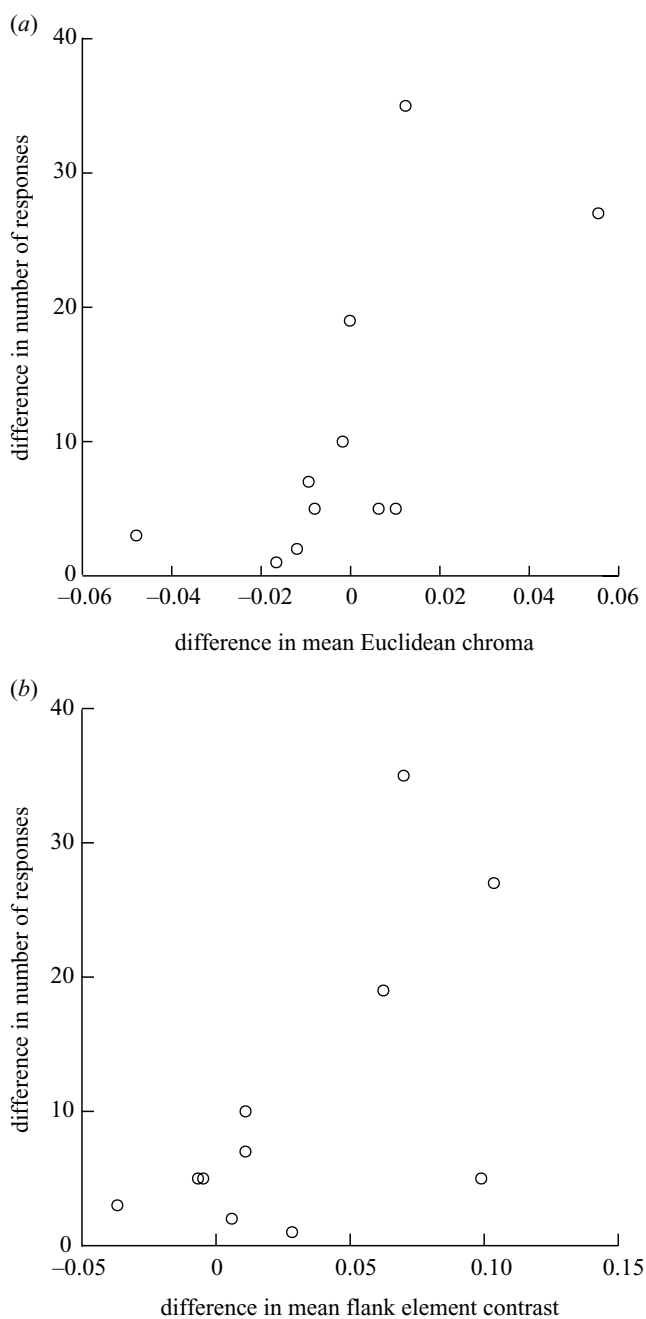


Figure 1. Regressions showing the effects of (a) the difference in mean Euclidean flank element chroma and (b) the mean Euclidean flank element contrast between opponent males on the difference in number of responses received from females.

$R^2 = 0.966$). The correlations between the chroma and contrast measures ranged from 0.178 ($p = 0.109$) to 0.526 ($p = 0.007$), with a mean adjusted R^2 of 0.352.

The reflectance data, which form the basis for the above calculations, also illustrate the differences between winners and losers. Figure 2 shows the mean reflectance of the upper flank (figure 2*a*) and lower flank (figure 2*b*), the two largest patches, for both winners and losers. The striking differences between the mean reflectances of winners and losers suggest that females prefer males that have more intense colours in these areas.

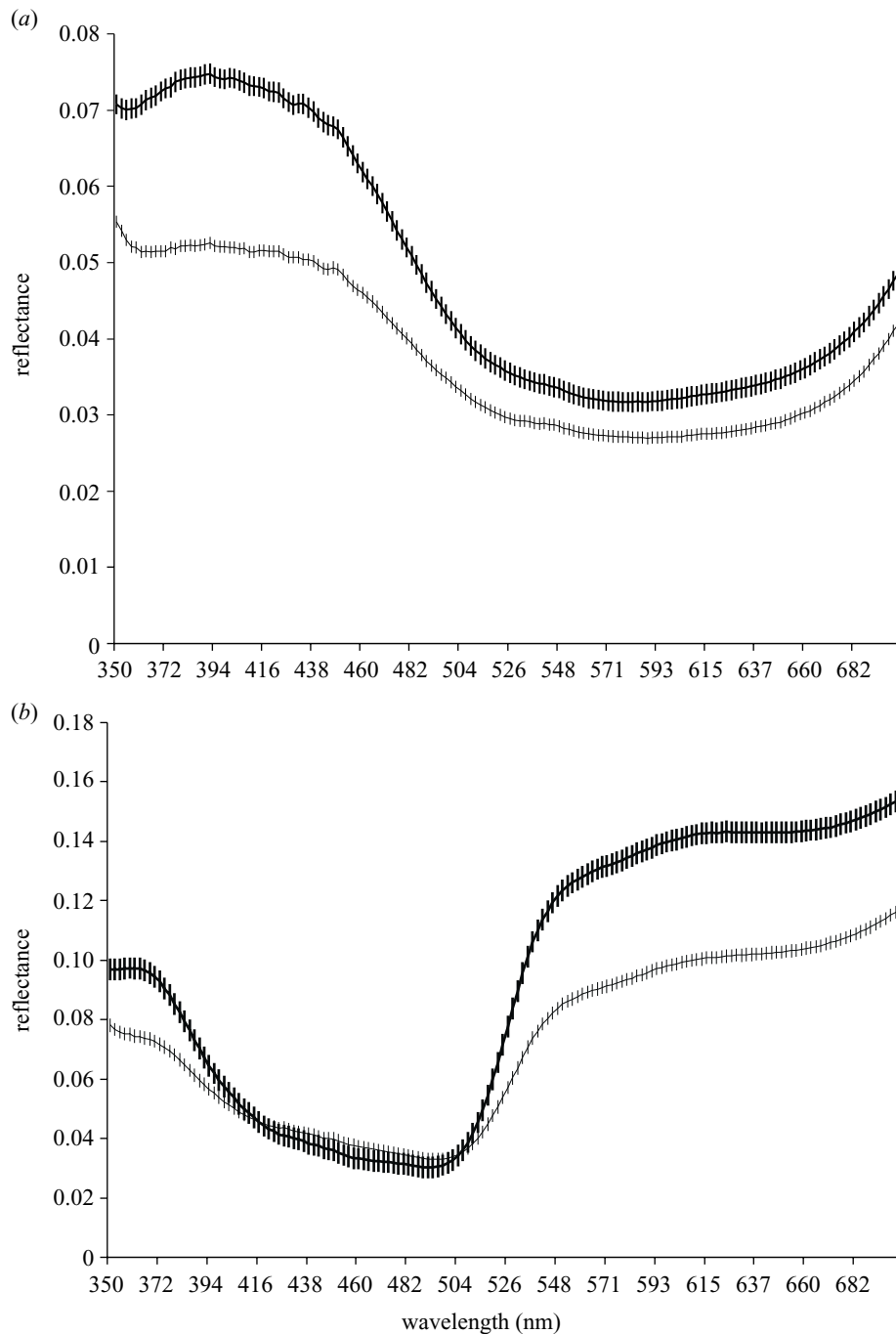


Figure 2. Mean reflectances for both winners (bold bars) and losers (narrow bars) of the (a) upper flank and (b) lower flank of Katala males; bars, ± 1 standard error.

4. DISCUSSION

Our results represent the first empirical evidence, to our knowledge, for directional sexual selection on male coloration within a haplochromine cichlid population. When given a choice between two males, female *L. fuelleborni* discriminate most strongly between those that differ substantially in colour saturation and within-pattern colour contrast. These two major results of our study were assessed using two separate perceptual analytical techniques, confirming the robustness of these results. This evidence supports over 20 years of claims that colour is a sexually selected characteristic in haplochromine cichlids, and may have played an important role in their astounding speciation events.

We should point out that, in some cases, a male who received more receptive responses did not necessarily have more saturated colours. This may be an artefact of the weightings of the different retinal system cones and cone pairs in our analyses. In the absence of additional data on visual physiology for our study species we assume equal weightings, but higher-level neural processing may weight receptors and their interactions differently (Endler 1990, 1991). Alternatively, this may be related to the effect of male display behaviour on female responsiveness, which is notoriously difficult to disentangle from other aspects of male attractiveness (Houde 1997). Our contrast results, however, showed a more consistent pattern of females preferring males with higher contrast among flank elements.

Chroma has been shown to be a sexually selected trait in several taxa, especially among fishes and birds (Baube *et al.* 1995; Brooks & Endler 2001; Hausmann *et al.* 2003). Whereas these saturated colours themselves may be some sort of 'handicap' or 'good genes' signal, an increase in the chroma of a colour further increases its potential contrast with other colours (Endler & Houde 1995). Therefore, we see a concomitant female preference in *L. fuelleborni* for males with high levels of contrast among the flank elements. The correlations among the measures of chroma and contrast, however, make it difficult to tease apart the separate effects of chroma and contrast on female choice with this dataset.

That we have demonstrated directional sexual selection on colour characteristics in a haplochromine cichlid gives further credence to models and theories predicting speciation via sexual selection in these fish. Preferences for males with more extreme chroma and contrast within the flank colour pattern elements potentially exclude less intensely coloured males, and their genetic contributions, from the breeding population of these fishes. With different hue preferences in different populations, this could act as a springboard to population divergence, resulting eventually in the appearance of new species in haplochromine cichlids. In future work it will be important to compare preference functions among populations together with their visual physiology of different populations (Boughman 2001).

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