Sexual selection on color and behavior within and between cichlid populations: Implications for speciation

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Abstract  Sexual selection is widely viewed as playing a central role in haplochromine cichlid speciation. Hypothetically, once divergent mate preferences evolve among populations of these fishes, reproductive isolation follows and the populations begin to behave as different species. Various studies have examined patterns of assortative mating among species and sometimes populations, but few have examined variation in directional preferences, especially among populations of the same species. We investigated mate choice behavior in two populations of Labeotropheus fuelleborni, a Lake Malawi endemic. We test whether mating preferences between populations are based on the same traits and in the same direction as preferences within populations. We examine the potential contributions of two classes of trait, color patterns and behaviors, to reproductive isolation. When females chose between either two males of their own population, or two from another, female preferences were generally similar (for the female population) across the two contexts. Mate choice patterns differed between (female) populations for a measure of color, but only modestly for male behavior. In a separate experiment we simultaneously offered females a male of their own population and a male from a different population. In these trials, females consistently preferred males from their own population, which were also the males that displayed more frequently than their opponents, but not necessarily those with color traits suggested to be most attractive in the previous experiment. Thus directional preferences for chroma and related aspects of color may be important when females are presented with males of otherwise similar phenotypes, but may play little role in mediating assortative mating among populations with substantially different color patterns. A preference for male behavior could play some role in speciation if males preferentially court same-population females, as we have observed for the populations studied herein [Current Zoology 58 (3): 475–483, 2012].

Keywords  Haplochromine cichlid, Sexual selection, Speciation, Color, Labeotropheus

In a recent review, Ritchie (2007) remarked that decisive evidence concerning the role of sexual selection in speciation is “surprisingly thin on the ground … the case for speciation occurring primarily by sexual selection is certainly not as well made as, say, the case for the preponderance of allopatric speciation.” Nevertheless the comparative evidence overall indicates that sexual selection facilitates speciation (Kraaijeveld et al., 2011) and case studies providing evidence of speciation by one process, sensory bias sexual selection, are beginning to accumulate (e.g. Boughman, 2001; Seehausen et al., 2008; reviewed by Mann and Seehausen, 2011). Theory provides a variety of mechanisms through which sexual selection can cause or facilitate speciation (e.g., Lande, 1988; Servedio, 2007; Uyeda et al., 2009, Van Doorn et al., 2009; reviewed by Kirkpatrick and Ravigne, 2002, Ritchie, 2007; Servedio et al., 2011); however theory also provides strong rationales for why sexual selection may rarely drive speciation by itself, especially in sympatry (reviewed by Ritchie, 2007). In the haplochromine cichlids of the African Great Lakes, preferences for conspecific mates have been suggested to arise as extensions of female preferences and/or sensory biases evolving rapidly within populations, whether by drift or selection, and potentially in any of allopatry, parapatry or sympathy (e.g. Turner and Burrows, 1995; Pauers et al., 2004; Seehausen et al., 2008). If this hypothesis is correct, then all else being equal, female cichlids should base within and between population mating decisions on the same traits and according to the same preference functions (sensu Basolo, 1990). For example, Allender et al. (2003) demonstrated that male nuptial color diversity in the Metriaclima zebra complex of Lake Malawi likely arose as a result of divergent selection among closely related, geographically proximate populations. Alternatively, preference functions between vs. within populations or species may bear little relation to each other and could potentially be evolving under different selection pressures (Mann and Seehausen, 2011).
Only recently have studies of female cichlid color preferences (thought to be key traits in cichlid speciation) moved beyond demonstrating different color patterns as a basis for assortative mating (Pauers et al., 2004; Maan et al., 2004) and additional detailed between population comparisons of preferences are needed (e.g., Seehausen et al., 2008, Maan et al., 2010). Many studies have compared the behavioral responses of females offered conspecific and heterospecific males (e.g., Holzberg, 1978; Seehausen, 1997; Seehausen and van Alphen, 1998; Verzijden and ten Cate, 2007), and some have even compared female investment (i.e., numbers of eggs laid) in conspecific and heterospecific mates (Knight and Turner, 2004; Kidd et al., 2006). Further, other studies have explored the possible use of non-visual cues by female haplochromine cichlids, and have found that olfactory (e.g., Plenderleith et al., 2005) and auditory (e.g., Amorim et al., 2004) cues play important roles in mate choice, and may contribute to speciation. These studies are important and have ascertained the boundaries between species as well as elucidating some aspects of assortative mating, yet few investigations have asked if the same preferences are expressed within and between populations, particularly in the allopatric or parapatric contexts that likely obtain for the vast majority of speciation events (e.g., Coyne and Orr, 2004).

Perhaps more surprisingly, the courtship behavior of male haplochromines has also been relatively little investigated. Possibly this is because in these fishes, male courtship is relatively stereotypical and conserved among species (McElroy and Kornfield, 1990). Typically, a male haplochromine will approach a female and engage in a lateral display. If the female is receptive, the male will intensify his behaviors, which become further and further suggestive of spawning, until the actual act occurs (Barlow, 2000). This sequence of interactions between males and females is common to almost all haplochromines, and generally thought not to be associated with divergence among populations or species, even among the African great lakes (McElroy and Kornfield, 1990; Seehausen, 1996). Nonetheless, the displays may be important to females selecting a mate, especially from within her own species and/or population, because these behaviors may be used to enhance the perception of other male characteristics, such as size, coloration, or other indicators of “quality” (Endler, 1995; Marshall et al., 2003; Barbosa and Magurran, 2006).

Here we compare patterns of female mate choice between two populations of *Labeotropheus fuelleborni* when presented with males of the same or different populations, to see whether the same or different preferences, measured in a proximal sense as female mate choice, are observed within and between populations and whether females from these populations use the same or different color traits when evaluating potential mates. Furthermore, we evaluate the influence of male courtship behavior on female mating decisions, asking again whether female preferences for male behavior have diverged and whether the same or different preferences are expressed within and between populations. We have previously documented assortative courtship preferences among these populations (Pauers et al., 2010) but have conducted only limited investigation of directional female preferences for particular traits (Pauers et al., 2004).

1 **Materials and Methods**

1.1 **Study organisms**

Individuals from two populations of *L. fuelleborni* were used in this study. The Katale population is located in the northwest region of Lake Malawi. These males feature a flank that is orange on the ventral portion and sky-blue on the dorsum. The dorsal fin is blue-white with a yellowish trailing edge. Katale females are grey or brownish-grey, sometimes with yellowish color on the dorsal fin. The Chipoka population is located in the southwest region of Lake Malawi. The males have a solid sky-blue body and a red dorsal fin. Chipoka females are uniformly grey. All experimental subjects were wild caught.

1.2 **Behavioral trials**

The behavioral arena was a 300 L aquarium divided into three sections: two side compartments approximately 30.5 cm long and a central compartment 61 cm in length. The sections were separated by permanent, clear, ultraviolet-transparent Plexiglas dividers. Thus, while non-visual cues were eliminated, or at least substantially impeded, the female had complete, unobstructed visual contact with each male. 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.

A total of 35 naïve females (13 Chipoka and 22 Katale) were used as subjects in a female choice experimental design. Females were only used when gravid to ensure they were responsive and that males would court them. A female in such condition was placed in the center compartment of the arena, and opaque Plexiglas dividers were placed in front of the transparent par-
tions. Two males were haphazardly selected from stock aquaria (Chipoka $n = 16$; Katale $n = 17$) and placed in the side compartments of the arena, one per compartment. Males were sometimes used twice in this experiment (Chipoka males: $n = 11$; Katale males: $n = 14$), but were only paired with the same opponent once (Kodric-Brown and Johnson 2003; Pauers et al. 2004; Pauers et al., 2010). The fish were then allowed to acclimate to the behavioral arena for a minimum of 4 hours before the observations began.

We conducted three types of experimental trials. The first two types placed a female in a setting in which she had to choose between two males of a single population. In the first of these, she was given a choice between males from her own population (“intra-population” i.e., a Katale female with two Katale males). In the second, she had to choose between males from the allopatric population (“inter-population” i.e., a Katale female with two Chipoka males). This yielded sample sizes of 7 Chipoka inter-population trios, 6 Chipoka intra-population trios, 12 Katale inter-population trios, and 10 Katale intra-population trios. Each female was used once in the first two sets of trials. In the third type, a subset of females ($n = 9$ Chipoka and $n = 8$ Katale) was used in trials in which they were given a choice between a Chipoka male and a Katale male presented simultaneously; these data were analyzed separately from the first two sets of trials (Table 1).

Table 1 Numbers and types of trials used in these experiments, including populations of the subjects

| Trial Type                  | Female Population | Male Population(s) | $n$ |
|-----------------------------|-------------------|--------------------|-----|
| Chipoka intra-population    | Chipoka           | Both Chipoka       | 6   |
| Chipoka inter-population    | Chipoka           | Both Katale        | 7   |
| Chipoka dual choice         | Chipoka           | 1 Chipoka, 1 Katale| 9   |
| Katale intra-population     | Katale            | Both Katale        | 10  |
| Katale inter-population     | Katale            | Both Chipoka       | 12  |
| Katale dual choice          | Katale            | 1 Chipoka, 1 Katale| 8   |

When an observation began, the opaque dividers were removed and a camcorder was started; no humans were present during the trials. After 45 minutes, the camcorder was stopped, the fish removed from the arena, and the males were weighed (to the nearest 0.01 gram), measured (total length; to the nearest .1 cm), and their reflectances collected (see below). The videotapes were scored later. We counted the number of lateral displays performed by each male, as well as the number of receptive responses given to each male by the female (sensu Seehausen and van Alphen, 1998; Pauers et al., 2004). For all subsequent analyses, a male was declared the “winner” of a trial if he received more receptive responses than his opponent; female preference was thus considered to be indicated by a greater number of receptive responses given to one of the opponent males.

1.3 Spectrophotometry

The methods used in this study have been described in detail in Pauers et al. (2004) and are only summarized here. Once a fish was removed from the arena, he was anaesthetized with a weak dose of MS-222 and then placed in an ice bath. Upon removal from the ice bath, the fish was placed on a black cloth and illuminated from its dorsal surface with four 50 W Solux halogen lamps and one 20 W ultraviolet blacklight. Using a quartz lens attached to an Oriel Instaspec IV CCD, measurements of reflected wavelengths were taken at eight points on the fish. These data were converted to actual reflectances by dividing them by measurements taken from a Spectralon white standard.

These reflectances were used to calculate a measure of chroma, or saturation of color as perceived by the conspecific viewer, a known sexually-selected characteristic in *L. fuelleborni* (Pauers et al., 2004). In the present analysis, we calculated $D_{mx}$, which is a measure of chroma based on the chromatic response functions of pairs of spectrally-adjacent retinal photoreceptors (Endler, 1991), for all eight spots on the fish. These eight individual $D_{mx}$ values were then summed to get a measure of overall chroma for each individual fish (Endler, 1991; Endler and Mielke, 2005). Each fish was photographed for color patch measurement after spectrophotometry, but during photography the fins typically folded and could not be reliably measured. Because the fins are conspicuous display elements, especially in the lateral display of male cichlid courtship (Barlow, 2000), and are likely especially so in the Chipoka population, we wanted to include their contribution to overall chroma; thus, we did not weight any color patch by its area. Other, sometimes more complex, visual models are available in the literature (reviewed by Endler and Mielke, 2005). We used this approach rather than more complex ones because it successfully predicted preferences in a previous study (Pauers et al., 2004); however, it should be noted that these calculations are limited by the use of a single set of vision data from a related species rather than population-specific data, and do not take into account backgrounds as they might occur in nature.

As the saturation of colors increases within and
among color patches, the chromatic contrast between and among these patches also often increases (Endler, 1991; Rush et al., 2003); chroma and contrast thus tend to be correlated with one another. In the Katale population of *L. fuelleborni*, chroma and contrast are thus correlated, so not only do females prefer males that have a greater mean chroma than their opponents, but also those with higher contrast among color pattern elements, whether calculated as a modified form of Endler’s D (Endler, 1990) emphasizing chroma, or as a Euclidean measure of color space contrast (Endler, 1991; Pauers et al., 2004). In preliminary analyses, we also considered the role Euclidean contrast might play in mate choice decisions between these two populations of *L. fuelleborni*. In these analyses, a difference in contrast between opponent males, in and of itself, was never found to be predictive of the difference in receptive responses distinct from effects of chroma/saturation; i.e. when both were included in an analysis contrast was always NS. We therefore have omitted contrast from the final models presented here.

### 1.4 Statistical analyses

The data were analyzed in Systat 10.0. The differences in the number of lateral displays performed and total chroma (as Dmx) between a pair of opponent males were used in linear models to predict the difference in receptive responses received by the opponent males; all differences were calculated as winner-loser; interactions with male population, female population, and both simultaneously were also considered for each difference. To better approximate normality, the behavioral variables (i.e., the difference in responses to males and the difference in displays to females) were square-root transformed as needed.

To examine patterns of female choice within and among populations, we utilized complete General Linear Models in which all main effects and any significant crossed effects were included; any non-significant crossed effects were excluded from our final models (Sokal and Rohlf, 1995). These analyses were conducted at two levels. In the first, we examined overall patterns of female choice, to determine whether or not there were female mate preferences that transcend population. In the second, we examined population-specific preferences for chroma and male display behavior.

A portion of the data presented herein was analyzed for a previous paper on the influence of aspects of male color on female receptive behavior (Pauers et al., 2004). The data published earlier consisted of results on color preferences, but not behavior, for one of the six population combinations presented here (Katale same population trios). Overall frequencies of male and female behavior in this experiment and assortative mating by population analyses, but not preferences for color or behavioral characters, were presented in Pauers et al. (2010).

### 2 Results

#### 2.1 Overall patterns of female choice between-males of one population

Of the four independent variables, and the possible two- and three-way interactions thereof, four were significant predictors of the difference in female response between opponents: the difference in the number of displays performed by opponent males; the interaction between female population and display number difference; the interaction between female population and the difference in chroma (as Dmx); and the interaction between male population and display number difference (Table 2). Thus, while females of both populations preferred males that displayed more frequently than their opponents, there were also differences between the populations in both color and display behavior preferences, as demonstrated by the significance of the Two-Way interactions.

#### Table 2 Results of GLM, including all main effects, on overall female preferences

| Effect                          | df | F    | P    |
|--------------------------------|----|------|------|
| Male Population                | 1  | 0.953| 0.338|
| Female Population              | 1  | 0.726| 0.402|
| Difference in Chroma (as Dmx)  | 1  | 0.759| 0.391|
| √ Difference of displays       | 1  | 36.437| <0.001|
| Male Population * √ display difference | 1 | 7.217 | 0.012 |
| Female Population * chroma difference | 1 | 6.906 | 0.014 |
| Female Population * √ display difference | 1 | 5.771 | 0.023 |
| Error                          | 25 |      |      |

All Females (*n* = 35, multiple $R^2$ = 0.694).

#### 2.2 Population-specific patterns of female choice

When analyzed separately, females of the Katale and Chipoka populations display distinctions in their preferences for male coloration and display behavior. The Katale females exhibited a preference for males, regardless of their population of origin, who displayed more than their opponent; for Katale females the difference between males in Dmx was also significant (Fig. 1; Table 3A).
Fig. 1  Patterns of preference for male coloration and behavior in females of the Katale population
A. Difference in chroma (as $D_{max}$) between opponent males. B. Difference in the number of displays performed.

Table 3  Female preferences for differences in display number and chroma between opponent males; all relevant main effects included in final model

| Effect                              | df | $F$   | $P$  |
|------------------------------------|----|-------|------|
| **A) Katale Females ($n = 22$; multiple $R^2 = 0.592$)*** |
| Male Population                     | 1  | 0.004 | 0.951|
| Difference in Chroma                | 1  | 11.329| 0.003|
| $\sqrt{\text{Display difference}}$ | 1  | 7.047 | 0.016|
| Error                               | 18 |       |      |
| **B) Chipoka Females ($n = 13$; multiple $R^2 = 0.832$)*** |
| Male Population                     | 1  | 0.982 | 0.351|
| Difference in chroma                | 1  | 0.543 | 0.482|
| $\sqrt{\text{Display difference}}$ | 1  | 24.068| 0.001|
| Male Population * $\sqrt{\text{Display difference}}$ | 1  | 6.861 | 0.031|
| Error                               | 8  |       |      |

In the Chipoka population, females had a nonsignificant preference for chroma differences between opponent males, though it is interesting to note that while they showed a trend toward increasing preference for chroma differences for Chipoka males, the preference was decreasing for Katale males (Fig. 2A; Table 3B). Like Katale females, Chipoka females too demonstrated an overall preference for males that displayed more frequently than their opponents. Also like Katale females (though significantly only for Chipoka females), they showed an especially strong preference for frequent male display when choosing between Katale males, as evidenced by the significant two-way interaction between display difference and male population. However, the significant interaction between display difference and female population in the comprehensive analysis results from their overall weaker (than Katale females, though still significant) preference for male display (Fig. 2B; Table 2, 3B; note figure axes not all too same scale).

2.3  Female choice between populations

In the final experiment, a female was offered one male from each population. We found in a previous analysis that females preferred the male from their own population (Pauers et al., 2010). When we asked if male chroma and/or display frequency were under sexual selection in this experiment, and might mediate both sexual selection and reproductive isolation, the difference in the number of displays between opponents was significant whereas chroma difference was not (Table 4; Fig. 3). Because interactions between chroma and display with female population were NS, the preference for males who display more vigorously is general in this final data set, whereas there is no evidence for either a general or population-specific female preference for chroma when choosing between populations.

3  Discussion

In both populations of *Labeotropheus fuelleborni* examined in these experiments, females consistently preferred males that displayed more frequently than their opponents; further, this preference was found whether the female was presented with two males of her own population, two males from a different population, or one male from her own population alongside a male from an allopatric population. However, when choosing between two males of a single population - comparing “apples with apples”-females of the Katale and Chipoka
Fig. 2 Patterns of preference for male coloration and behavior in females of the Chipoka population
A. Difference in chroma (as $D_{max}$) between opponent males. B. Difference in the number of displays performed; only significant trendlines are shown.

Table 4 Female preference for differences in chroma and male display behavior when offered a choice between both a Katale and a Chipoka male

| Effect                          | df | $F$  | $P$  |
|---------------------------------|----|------|------|
| Female Population               | 1  | 3.932| 0.069|
| Difference in Chroma            | 1  | 0.838| 0.377|
| Difference in display #         | 1  | 18.084| 0.001|
| Error                           | 13 |      |      |

All females ($n = 17$; multiple $R^2 = 0.722$)

Fig. 3 Preference for male behavior when offered a Chipoka and a Katale male simultaneously

populations diverged from each other in their preferences with regard to male coloration. In that context, Katale females most preferred those individuals, no matter their population of origin, who had higher $D_{max}$ values than their opponents, while Chipoka females did not demonstrate any significant preference for differences in chroma between opponent males.

It should be noted that, while increasing chroma was found to be a significant predictor of female choice, a male who received more receptive responses did not necessarily have a higher $D_{max}$ than his opponent. This may represent an artifact 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 *L. fuelleborni* (including potential variation among populations), we assumed that all retinal cones were equally weighted by the visual system, but higher-level neural processing may weight receptors and their interactions differently (Endler, 1990, 1991). This could also be related to the effect of male display behavior on female responsiveness, which is notoriously difficult to disentangle from other aspects of male attractiveness (Houde, 1997), or to females varying greatly in preference strength, with choosy females more consistently preferring high chroma males - this point is discussed further in Pauers et al. (2004), Braithwaite and Barber (2000) and Pauers et al. (2004).

In the haplochromine cichlids of the African Great Lakes, male courtship behavior is remarkably consistent among species, and even among species from different lakes (McElroy and Kornfield, 1990; Seehausen, 1996). Thus, it is not surprising that females of both populations preferred males who displayed more frequently than their opponents. Further, given that this preference was consistent no matter the combination of males offered, this trend is a fuller demonstration that male dis-
play behavior, and the concomitant female preference for male display, is a plesiomorphic character among populations and species of African haplochromines. This result is also consistent with a previous analysis of patterns of mate choice between these populations, which found that males display more frequently to females from their own populations (Pauers et al., 2010). This raises the possibility that high male display rates in these experiments are in part a response to attention from a female, though it should be noted that the cause-and-effect relationship between male display and female attention in these fishes is not well understood, and that experimentally controlling for male behavior, while desirable, is difficult (Houde, 1997; Maan et al., 2004; Maan et al., 2010; Pauers et al., 2010).

The divergent preferences for male coloration between the Katale and Chipoka populations of *L. fuelleborni* are not readily explained. The Katale males have a relatively complex color pattern that is highly dependent upon contrast among color pattern elements. Further, this contrast is correlated with chroma, so that males with high levels of contrast also have high levels of chroma (Pauers et al., 2004). Our current finding of Katale preferences based on male $D_{ax}$ are consistent with our previous findings that Katale females prefer sympatric males with higher levels of weighted contrast and chroma (Pauers et al., 2004), but our finding that this preference carries over into Katale female preferences for Chipoka males is surprising. It suggests that a preference for saturated colors may be hard-wired in this population, at least when females are presented with males of otherwise similar phenotype; in other words, when presented with a choice involving males not of her own population, a Katale female still uses the decision rules she would use when deciding amongst Katale males.

Preference for male coloration in Chipoka females is less clear-cut. The contrast between the bright red dorsal fin and the blue body seems like an important signal that would be emphasized during a lateral display; furthermore, the contrast between these patches would be maintained even in the absence of a long wavelength-sensitive photoreceptor (Pauers, 2011). Perhaps the use of patch area-weighted contrasts would have revealed this effect. In addition, our understanding of mating preferences will improve further as sensory data accumulate and our ability to model the perception of these fishes improves; we would like, for example, to have species and population-specific visual physiology data for our study system. It is also possible that the trends we observed would be significant with a larger sample, or that color pattern in this population functions mainly in a different context, perhaps male-male interactions.

In settings in which females were offered a choice between a Katale and a Chipoka male, it is somewhat surprising that differences in chroma between these opponents did not have a straightforward effect on the differences in female receptive responses. These results suggest that assortative mating by population is not a simple extension of within-population (even within a different population) color preferences in this system or Katale females would likely prefer Chipoka males, who tend to have overall higher chroma. Since the male nuptial color patterns of these populations are so strikingly different, it is possible that females recognized same-population males using only cues from the color pattern (i.e., the sizes, relative positions, and hues of color pattern elements), and ignored more subtle cues like differences in chroma between opponents. Further, the ability of males to recognize same-population males, and accordingly modulate their display activities (Pauers et al., 2010), may have also played a role in determining female mate choice. Thus, despite predictions to the contrary (summarized in Barlow, 2000), male *Labeotropheus* may also contribute to reproductive isolation by preferentially displaying to females of their own population. It is interesting, then, that a plesiomorphic preference for high levels of male display activity can not only mediate selection on male mating success, but might also act in combination with assortative courtship by males to generally enhance assortative mating among populations and/or species. Independent manipulations of color patterns and the behavior of both males and females would be required to definitively disentangle these effects.

Our results further suggest differing roles in mate choice for different male characteristics. While vigorous male display behavior is probably universally preferred by all females (McElroy and Kornfield, 1990; Seehausen, 1996; Pauers et al., 2010), it is likely that gross color pattern acts as an important “identifier” of species and/or population. Given that most published studies of mate choice in haplochromine cichlids have involved choices between very differently-colored males (e.g., Seehausen, 1997; Couldridge and Alexander, 2002; Knight and Turner, 2004; this study), it is likely that females are responding to these differences in male color pattern among opponents (Dalton et al., 2010; Pauers, 2011); indeed, when color pattern differences between opponent males are subtle, assortative mating...
tends to break down (Egger et al., 2010). Populations may also differ in preferences for finer scale attributes of color, like chroma, without these preferences necessarily mediating patterns of reproductive isolation. Our current analysis has confirmed and unexpectedly extended a previous result (Pauers et al., 2004), that females of the Katale population of L. fuelleborni prefer males with more saturated colors than their opponents, showing that this preference extends to non-Katale conspecific males. Additionally, the current study has found that females of the Chipoka population have no detectable preference for saturated colors, and that this lack of preference is also consistent, no matter the type of male offered.

Somewhat surprisingly, the available evidence suggests that sexual selection on color intensity appears to function only under certain circumstances in Labeotropheus. When offered two males from the same population, females seemed more likely to engage in comparisons of traits like the saturation of, and contrast between, colors. On the other hand, when two different males were offered, and assortative mating could be based on obvious differences in male color pattern, some preferences that are important when males are otherwise similar appear to play a diminished role. Thus, while sexual selection may function within a population of Labeotropheus, and may yet be the wellspring of divergence (e.g., Pauers et al., 2004), once male nuptial color differences are fixed among populations, female preferences for other aspects of color pattern may play little role in assortative mating. In future work it will be important to better elucidate the visual physiology of this species and the extent to which it varies amongst population (e.g. Seehausen et al, 2008), to assess the robustness of these findings.

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