Differences among reciprocal hybrids of *Labeotropheus*

Michael J. Pauers · Jacob Hoffmann · Leah Jiang-Bo Ackley

Received: 19 March 2022 / Revised: 4 November 2022 / Accepted: 12 November 2022 / Published online: 22 November 2022
© The Author(s), under exclusive licence to Springer Nature Switzerland AG 2022

Abstract Current evidence suggests that hybridization played a crucial role in the early evolution and diversification of the species flocks of cichlid fishes in the African Great Lakes. Nonetheless, evidence for hybridization in the extant cichlid fauna is scant, suggesting that hybridization is rare in the modern era, perhaps enforced by natural or sexual selection acting against F1 hybrids. Additionally, most experimental studies of hybridization perform a hybrid cross in one direction, ignoring the reciprocal hybrid. In this study, we perform reciprocal crosses between sympatric congeners from Lake Malawi, *Labeotropheus fuelleborni* and *L. trewavasae*, in order to compare the body shape and coloration of males of both of these hybrids, as well as to examine how these hybrids fare during both inter- and intrasexual interactions. We found that *L. trewavasae*-sired hybrid males are intermediate to the parental species both morphologically and chromatically, while the reciprocal *L. fuelleborni*-sired hybrids are likely transgressive hybrids. Males of these transgressive hybrids also fare poorly during our mate choice experiments. While female *L. trewavasae* reject them as possible mates, male *L. trewavasae* do not make a distinction between them and conspecific males. Selection against transgressive F1 hybrids as observed in our crossing experiments may help explain why contemporary hybridization in Lake Malawi cichlids appears to be rare.

Keywords Cichlids · Lake Malawi · Hybridization · Transgressive phenotype · Mate choice · *Labeotropheus*

Introduction

Until recently, hybridization was not viewed as a significant source of adaptive variation in animals. This is partially due to the long-standing view that
hybridization acts as a destructive force, leading to maladapted “dead ends,” but also due to the fact that there were very few convincing examples of adaptive introgression in animals (Mayr, 1963; Stelkens et al., 2009; Hedrick, 2013). Some of the best recent evidence for this phenomenon in vertebrates comes from the radiations of cichlid fishes in the African Great Lakes (e.g., Gante et al., 2016; Koblmuller et al., 2017; Meyer et al., 2017). These fishes represent the largest and most diverse radiations of vertebrate animals in the world, and have been rich study systems for evolutionary biologists, providing insights into speciation, adaptive radiation, and the role sexual selection can play in both of these processes (Barlow, 2000; Kornfield & Smith, 2000). Experiments involving controlled hybridization between species have provided evidence of morphological (e.g., McElroy & Kornfield, 1993; Albertson & Kocher, 2001; Stelkens et al., 2009), chromatic (e.g., Brzozowski et al., 2014; Albertson et al., 2014; Selz et al., 2014a), behavioral (Feller et al., 2020), and ecological (Selz & Seehausen, 2019) novelty. A study of hybrid novelty using species thought to be ancestral to the extant radiations in Lakes Tanganyika, Malawi, and Victoria found that inbred F2 individuals had a significantly increased range of morphological variation compared to that of both the parental and F1 generations (Selz et al., 2014b). This finding indicates that hybridization, far from constraining evolution, could be an important source of phenotypic novelty, especially under the ecological and environmental conditions in which these radiations occurred (e.g., Danley et al., 2012; Ivory et al., 2016; Santos-Santos et al., 2021). For example, after finding geological evidence of significant lake level fluctuations throughout the history of the Lake Malawi basin, Ivory et al. (2016) hypothesize that hybridization during times when the lake was shallow (“green lake” conditions) was an important source of phenotypic novelty that helped the cichlid flock radiate into the larger array of habitats and trophic niches available when lake levels subsequently rose (“blue lake” conditions), a scenario that has significant phylogenetic support (Genner & Turner, 2012, 2015).

While the above-listed studies powerfully demonstrate how hybridization could act to produce phenotypic variation during the early stages of adaptive radiation in the cichlids of the African Great Lakes, they do not directly address the fate of hybrids in the extant cichlid communities of these lakes. Pauers et al. (2018) found that F1 hybrids of Labeotropheus fuelleborni and L. trewavasae were less efficient feeders than either of the parental species, leading to the expectation that such hybrids would not be successful in the wild. Additionally, many of these species are notoriously assortative with regards to mate choice, except under anthropogenically altered conditions (Seehausen et al., 1997; Egger et al., 2012), and evidence of hybridization in the wild is exceedingly rare (Stauffer et al., 1996; Smith et al., 2003). Furthermore, F1 hybrids are uncommon in the extant lacustrine radiations, and are unlikely to have much reproductive success (Crapon de Caprona, 1986; Ribbink, 1990).

The fate of hybrid cichlids during sexual selection has not often been studied. In the few available studies examining patterns of mate choice involving hybrids (e.g., Svensson et al., 2011, 2017; Selz et al., 2014a), only female choice, examined post hoc via molecular genetic assessment of parentage, was evaluated; assessments of female mate choice behavior, via receptive responses or numbers of visits (e.g., Pauers et al., 2010; Pauers & Grudnowski, 2022) have rarely been conducted (e.g., Haesler & Seehausen, 2005). While more involved and time-consuming to evaluate, direct observations of courtship and receptive behavior are important due to their potential additional insights on the degree of behavioral resistance to introgression (e.g., Barlow, 2000, 2002; Coyne & Orr, 2004). Additionally, studies of intrasexual aggression, comparing the responses of territorial males to conspecific and hybrid males, have not been conducted. Given the importance of territory acquisition to male fitness for many of these species (Ribbink, 1990; Barlow, 1993; Rometsch et al., 2021), exploring this would provide valuable insights into how hybrid males would fare in a natural population.

Also missing from current studies of hybridization in cichlids are comparisons of F1 offspring produced via reciprocal crosses between the parent species. In the relatively few studies in which reciprocal crosses were made, the resulting hybrid progeny were pooled in the subsequent analyses, thus obscuring their parentage (e.g., Husemann et al., 2017). Given the roles sexual conflict and chromosomal sex determination play in determining the phenotype (e.g., Roberts et al., 2009), it seems especially important to compare hybrid offspring from reciprocal crosses.
The genus *Labeotropheus* Ahl 1927 is a small genus of haplochromine cichlids endemic to Lake Malawi, the species of which have been crucial in studies of the role of hybridization in the evolution of phenotypic novelty this species flock (e.g., Albertson & Kocher, 2001; Albertson et al., 2014; Concannon & Albertson, 2015). Hybrids of *Labeotropheus fuelleborni*, in particular, have been used to elucidate both evolutionary development and adaptive introgression in numerous phenotypic traits. In the above-mentioned study of feeding performance in hybrid *Labeotropheus*, Pauers et al. (2018) produced experimental F1 hybrids of *Labeotropheus* by crossing male *L. trewavasae* with female *L. fuelleborni*, but did not produce reciprocal hybrids. These hybrids, although they had a distinct body shape, were most morphologically similar to the maternal species, *L. fuelleborni*, and the color pattern of the male hybrids was most similar to that of male *L. fuelleborni* (Pauers et al., 2018; Pauers, pers. obsv.). We thus wanted to conduct a comparative study of body shape and male coloration in the parental species and their reciprocal hybrids; we were especially curious whether such *L. fuelleborni*-sired hybrids would also be most phenotypically similar to their maternal species, or if they would display a different, perhaps transgressive, morphological and chromatic phenotype. Transgressive hybrids, those that have a phenotype outside of the combined range of their parental species, are of particular interest to evolutionary biologists, as they represent a potential source of adaptive novelty (Holzman & Hulsey, 2017; Husemann et al., 2017). Additionally, we were interested in how these hybrids would fare in behavioral contexts, specifically intra- and inter-sexual selection.

Methods

Experimental animals

Wild caught adult specimens of *Labeotropheus fuelleborni* and *L. trewavasae* were obtained from a reputable Malawi-based exporter of cichlid fishes (Stuart M. Grant, Ltd.); both species were captured at the Chidunga Rocks in southwestern Lake Malawi. Twenty individuals of each species were obtained, ten of each sex. These adults were segregated by species and sex into four separate 160 l aquaria. Fishes were fed to satiation daily with a mixture of spirulina flake food (e.g., Formula Two; Ocean Nutrition, Newark, CA, USA), cichlid pellets (e.g., Hikari Cichlid Excel; Kyorin Co., Ltd., Hyogo, Japan), and a variety of frozen foods (e.g., bloodworms, brine shrimp; various manufacturers). Water temperature was maintained at 25°C, and water quality was maintained using standard box filters and biweekly 50% water changes.

Intraspecific F1 individuals of both species were produced by moving randomly selected groups of adults (1–3 males with 2–5 females) into separate 160 l aquaria, which were maintained as described above. The breeding groups of fishes were fed to satiation daily with the assortment of foods provided to the fishes in the stock tanks. Mouthbrooding females were isolated in separate aquaria (80 l or 120 l) until their fry were released at about 21 days post-fertilization. Using this same breeding and rearing protocol, two different hybrids were produced via reciprocal crosses between *L. fuelleborni* and *L. trewavasae* by pairing 1–3 males of one species with 2–5 females of the other.

All F1 individuals, both the two intraspecific treatment groups (e.g., *L. fuelleborni* and *L. trewavasae*) and the interspecific hybrids (*L. fuelleborni*-sired hybrids and *L. trewavasae*-sired hybrids), were raised to adulthood under the same aquarium conditions under which the parental generation was maintained. Additionally, since all of these F1 individuals were fed the same diet, we were able to control for possible phenotypic plasticity in trophic anatomy and morphology (e.g., van Snick Grey & Stauffer, 2004). Once they were full grown and sexually mature, these F1 fishes were later used in the experiments described below.

For the morphometric and spectrophotometric analyses described below, we only used sexually mature males of each of the four treatment groups (*L. fuelleborni*, *L. trewavasae*, *L. fuelleborni*-sired hybrids, and *L. trewavasae*-sired hybrids). There is great sexual dimorphism in color pattern in these species, and given the role male color pattern plays in sexual selection in the *Labeotropheus*, we limited our analyses to the males (Pauers et al., 2004, 2008; Pauers & McKinnon, 2012). Additionally, the coloration of male cichlids from Lake Malawi tends to be more modular (i.e., consisting of several distinct biochemical, structural, or anatomical components, or modules; Klingenberg, 2009) and thus more
evolutionarily labile than that of female coloration, which is considered to be integrated (i.e., comprised of interconnected, covarying components; Klingenberg, 2009; Brzozowski et al., 2014; Albertson et al., 2014), and would be more likely to show variation from the parental species than female coloration.

Since we limited the investigations of coloration to sexually mature males, we similarly limited our investigations of body shape to these same males, so that we would have congruent and complimentary data for each specimen in the analyses.

Many of our female *L. fuelleborni* had become reproductively senescent, and our attempts to obtain new *L. fuelleborni* stock, especially females, were hampered by a lack of *L. fuelleborni* from Chidunga Rocks in the U.S. aquarium trade, as well as a near cessation of imports of wild fishes from Lake Malawi due to shipping and supply chain issues caused by the COVID-19 pandemic. This limited our behavioral experiments to those involving *L. trewavasae* males and males resulting from the cross of a *L. fuelleborni* male and a *L. trewavasae* female.

**Geometric morphometrics**

Sexually mature males of each of the four treatment groups were photographed with a digital camera under fluorescent room lighting. The fishes were lightly anesthetized with MS-222 (approximately 25 mg/l), then removed from the MS-222 bath and placed on a black cloth such that the fish’s left flank was facing up towards the camera; additionally, the fish’s dorsal, anal, and pelvic fins were pinned in their erect positions. After the photographs were taken, the fish were transferred to a warm (28°C), aerated aquarium in which they were allowed to recover from the MS-222 before being returned to their stock aquarium. The photographs were imported into ImageJ, and 19 landmarks, following Pauers and McMillan (2015) and Pauers et al. (2018), were digitized from each specimen; see Fig. 1a for a picture and description of the landmarks.

The morphometric analyses followed Pauers et al. (2018). Briefly, the (x, y) coordinates of each landmark for each specimen were subjected to a Generalized Procrustes Analysis in the program Coordgen8 (Sheets 2001) to reduce the effects of size, orientation, and position in the comparison of shape. Next, a multiple regression of shape on geometric centroid size was performed using the Standard6 software which eliminates shape differences due to size. These data were then subjected to a thin-plate spline procedure to generate geometric descriptors of shape variation called relative warps (RW); this procedure

![Fig. 1](image-url)
was performed using the program TPSRelW (version 1.73; Rohlf, 2021). All deformation grids were generated with TPSRelW. We used Systat 10.0 (Systat Software, Inc.; Palo Alto, California, USA) to perform a MANOVA in which treatment group (e.g., *L. fuelleborni*, *L. trewavasae*, *L. fuelleborni*-sired hybrid offspring, and *L. trewavasae*-sired hybrid offspring) was used as the independent variable, and the RW scores were used as dependent variables; we then conducted Bonferroni-corrected pairwise tests among treatment groups for each RW. We also used Systat 10.0 to create bivariate plots of the RW scores.

Spectrophotometry

Adult males of all four treatment groups were placed individually in a weak solution of MS 222 (approximately 25 mg/l) and removed when the subject’s respiration slowed and it lost equilibrium; anesthetized fishes were then transferred to an ice bath (Pauers et al., 2004). After removal from the ice bath, fish were placed on a piece of black cloth underneath a UV-transmitting quartz lens mounted on a macro tube, which directed light via a fiber-optic cable into an Oriel Instaspec IV CCD and illuminated the fish from its dorsal surface with a Newport 100 W ozone-free xenon lamp, a 385 nm LED flashlight, and a 15 W fluorescent blacklight. We obtained reflectance data from six spots on each fish (Fig. 1b); the recorded wavelengths were converted to actual reflectance measurements by dividing them by analogous measurements taken from a Spectralon white standard. After the measurements were made, the fish were transferred to a warm (28°C), aerated aquarium in which they were allowed to recover from the MS-222 before being returned to their stock aquaria.

Numerous measures of brightness, chroma, and hue were extracted from the reflectances using the pavo package (version 2.7.1) in R (Maia et al., 2019), and a principal components analysis (PCA) was performed on these chromatic variables in Systat 10.0 (Systat Software, Inc.; Palo Alto, California, USA). We then conducted an ANOVA in which treatment group was used as the independent variable and the principal components were dependent variables to determine whether the factor scores of our four treatment groups were statistically different; we additionally conducted Bonferroni-corrected pairwise tests among treatment groups for each principal component.

Additionally, we were interested in the degree to which the color patterns of these treatment groups might be modular versus integrated; modular phenotypes consist of distinct, independent components; integrated phenotypes consist of interconnected, covarying components (e.g., Klingenberg, 2009). Male cichlids from Lake Malawi typically have coloration that is more modular, and thus more evolutionarily labile and responsive to sexual selection than the typically canalized and integrated color patterns of females (sensu Brzozowski et al., 2014; Albertson et al., 2014). We used the Principal Components Approach suggested by Brzozowski et al. (2014) and Albertson et al. (2014) to investigate the degree to which the color patterns of these fishes are integrated. We thus conducted principal components analyses separately for each treatment group using the same set of spectral characteristics as when all fishes were pooled, and examined the number of eigenvalues > 1, as well as the percent of variance explained by each principal component axis.

Sexual selection

We performed two experiments on sexual selection involving the two types of males available. The first of these experiments examined the aggressive reactions of male *L. trewavasae* to two opponents presented simultaneously, one male *L. trewavasae*, and one *L. fuelleborni*-sired hybrid male. Ten male *L. trewavasae* (total length: mean = 11.43 ± 0.63 cm) were the focal subjects, and ten unique pairs of opponents were created from five male *L. trewavasae* (total length: mean = 10.58 ± 0.47 cm) and five male *L. fuelleborni*-sired hybrids (total length: mean = 10.60 ± 0.28 cm). Following the methods of Pauers and Grudnowski (2020), the focal male *L. trewavasae* was placed in the central compartment of a three-part arena and allowed to acclimate for a period of 24–48 h; see Pauers and Grudnowski (2020) for a picture of the arena. On the day of the experiment, the two stimulus males were placed in two smaller Plexiglas aquaria that were suspended on opposite ends of the arena, and the fish were allowed to interact for ten minutes. The interactions were recorded on a digital video camera, and after the ten minute interaction period, the camera was stopped, and all individuals were removed.
from the arena and measured for total length and had their eggspots counted before being returned to their respective cohort tanks.

We then reviewed the recording, counting the numbers of four aggressive behaviors (i.e., butts, bites, lateral displays, and frontal displays) the focal male directed to each opponent, as well as the sum of these four behaviors (“total aggression”). We specifically tested the hypothesis that the focal male *L. trewavasae* would direct more aggressive behaviors towards conspecific males, as found in Pauers and Grudnowski (2020). These data were checked for normality and analyzed using the appropriate (i.e., parametric versus nonparametric) statistical tests in Systat 10.0.

In the second experiment, we examined female mate choice in female *L. trewavasae* when offered a conspecific male and a *L. fuelleborni*-sired hybrid male, testing the hypothesis that females should direct more responses to conspecific males. The experimental arena consisted of a filtered, heated 132 L aquarium maintained at the same parameters as the cohort aquaria. Two semi-permanent, ultraviolet-transparent Plexiglas dividers were installed 30.5 cm from each side of the aquarium; these barriers demarcated the male territories, which had a ground area of 930.25 cm² and a volume of about 28.4 L. Each male was given a single brick (15.25 cm × 15.25 cm × 5 cm) which served as a territory and shelter; each brick was positioned such that a fish could hide behind or beside it and not be readily visible to the other fishes. Immediately in front of both clear dividers were removable opaque dividers, which allowed the fish to acclimate to the arena simultaneously without being in visual contact with one another. The centre portion of the arena, with a ground area of 1859.3 cm² and a volume of about 56.7 L, was the female’s territory; the female was also provided a small shelter in the form of a brick (15.25 cm × 15.25 cm × 5 cm) and an artificial plant.

A trio of fishes, a gravid female and two males, were randomly selected from their cohort aquaria and moved to the arena. Ten female *L. trewavasae* (total length: mean = 10.9 ± 0.20 cm) were the focal subjects, and ten unique pairs of opponents were created from five male *L. trewavasae* (total length: mean = 11.96 ± 0.66 cm; anal fin eggspots: mode = 3) and five male *L. fuelleborni*-sired hybrids (total length: mean = 10.94 ± 0.20 cm; anal fin eggspots: mode = 3). The female was placed into the larger, central compartment and each male was placed separately into one of the smaller male territories. The fish were allowed to acclimate to the experimental arena for a period of 48 h. After the 48-h acclimation period, the opaque dividers were removed and the interactions between the female and both stimulus males were recorded using a video camera for 20 min with no humans present. After 20 min, the recording was stopped, and all individuals were removed from the arena and measured for total length and had their eggspots counted before being returned to their respective cohort tanks.

The video recordings were then reviewed, counting the number of times a female directly approached a male on the opposite side of the transparent barrier (Pauers & Grudnowski, 2022). The number of visits to a given male corresponds to the amount of time spent near that male, which is known to be indicative of male choice in the rock-dwelling cichlids of Lake Malawi (Couldridge & Alexander, 2001). These data were checked for normality and analyzed using the appropriate (i.e., parametric versus nonparametric) statistical tests in Systat 10.0.

**Results**

**Experimental animals**

Our experimental crosses produced 12 *L. trewavasae*-sired hybrid males and seven *L. fuelleborni*-sired hybrid males that survived to adulthood (Fig. 2). While many of the female *L. fuelleborni* were responsive to the male *L. trewavasae* and produced at least some hybrid offspring (e.g., Pauers et al., 2018), we noticed that the female *L. trewavasae* were resistant to male *L. fuelleborni*, and only two female *L. trewavasae* successfully spawned with a heterospecific mate (Pauers, pers. obsv.). We then selected 12 adult male *L. fuelleborni* and 12 adult male *L. trewavasae* from our cohort stocks for the morphometric and spectrophotometric analyses.

**Geometric morphometrics**

The relative warps analysis found significant differences in morphology among the four treatment groups of *Labeotropheus*. Relative Warp 1 explains
25.07% of the variation in body shape and differentiates a deeper body and upturned neurocranium and snout, as seen in *L. fuelleborni* and the *L. fuelleborni*-sired hybrids, from a slender body with a straighter head and horizontal snout as seen in *L. trewavasae* and the *L. trewavasae*-sired hybrids. Relative Warp 2 explains 14.58% of the variation in shape among our specimens, and differentiates the prominently upturned neurocranium and snout of the *L. fuelleborni*-sired hybrids from the head profiles found in the other three treatment groups (Fig. 3).

In Fig. 3, it is interesting to note that *L. trewavasae*-sired hybrids have a body shape that is similar to that of *L. trewavasae*. Along both Relative Warps 1 and 2, these hybrids are significantly different from *L. fuelleborni*, though not from *L. trewavasae* (Table 1; Supplementary Table 1). The *L. fuelleborni*-sired hybrids, on the other hand, are significantly different from all of the other treatment groups of *Labeotropheus* along Relative Warp 1, but are only significantly different from the *L. trewavasae*-sired hybrids along Relative Warp 2 (Table 1; Supplementary Table 1).

**Spectrophotometry**

The average reflectances of each treatment group of *Labeotropheus* for each of the six patches measured are shown in Fig. 4. These reflectance curves illustrate the chromatic differences among the treatment groups, especially the *L. fuelleborni*-sired hybrids. These hybrids have strong ultraviolet reflectance in their caudal peduncle, operculum, and ventrum, with a peak at about 350 nm, but lack the additional peak at 400 nm found in the other three treatment groups (Fig. 4). Interestingly, a somewhat inverse pattern is found in the dorsum and forehead reflectances, where the *L. fuelleborni*-sired hybrids lack a strong ultraviolet component to their coloration, especially in comparison to *L. trewavasae*, but have a strong peak at about 425 nm (Fig. 4). The reflectance of the dorsal fin distinguishes *L. trewavasae* from the other three treatment groups, with its unique broad peak throughout the ultraviolet and violet wavelengths, but lack of a subsequent and relatively less intense peak in the longer wavelengths as found in *L. fuelleborni* and both hybrids (Fig. 4).

The principal components analysis performed on all of the various measures of brightness, chroma, and hue reveals further distinctions among the four treatment groups. The loadings for the first two principal components are shown in Table 2. The first axis, Color PC 1, accounts for 19.35% of the variation in color, and discriminates between the high and narrow-peaked ultraviolet reflectance of the caudal peduncle and ventrum found on the *L. fuelleborni*-sired hybrids, as well as the more bimodal, ultraviolet and violet caudal peduncle and ventrum of the other three treatment groups (Fig. 5). The second principal component, Color PC 2, accounts for 16.97% of the variation in color, and distinguishes the unusual dorsum and operculum reflectances of the *L. fuelleborni*-sired hybrids from the other treatment groups (Fig. 5).

An ANOVA of the scores of Color PCs 1 and 2 for each treatment group indicates significant differences among the treatment groups for both (Table 3). The
L. fuelleborni-sired hybrids are significantly different from the other three groups along both Color PC 1 and 2 (Table 3). The L. trewavasae-sired hybrids are significantly different from L. trewavasae along both, but not from L. fuelleborni (Table 3).

Exploring modularity and integration in the pigmentation of the four treatment groups using the approach of Brzozowski et al. (2014) and Albertson et al. (2014) revealed that, unlike the other three treatment groups, the pigmentation of the L. fuelleborni-sired hybrid males is consistent with an integrated color pattern. For both parental species, nine eigenvalues were > 1, with the first axis accounting for 29.81% of the variation in L. fuelleborni, and 30.20% in L. trewavasae. The L. trewavasae-sired hybrids had 10 eigenvalues > 1, and the first principal component axis explained 29.73% of the observed variation.

| Variable | Sum of squares | df | Mean square  | F     | P       |
|----------|----------------|----|-------------|-------|---------|
| RW 1     | 0.007          | 3  | 0.002       | 20.829| ≤0.001  |
| Error    | 0.004          | 34 | 0.000       |       |         |
| RW 2     | 0.002          | 3  | 0.001       | 4.718 | ≤0.01   |
| Error    | 0.004          | 34 | 0.000       |       |         |
| RW 3     | 0.002          | 3  | 0.001       | 7.131 | ≤0.001  |
| Error    | 0.004          | 34 | 0.000       |       |         |
| RW 4     | 0.001          | 3  | 0.000       | 5.908 | ≤0.01   |
| Error    | 0.004          | 34 | 0.000       |       |         |
| RW 5     | 0.000          | 3  | 0.000       | 0.039 | 0.989   |
| Error    | 0.004          | 34 | 0.000       |       |         |

Relative Warps 6–34 were ns; data not shown. Results of pairwise comparisons of treatment groups can be found in Supplementary Table 1.
Finally, the *L. fuelleborni*-sired hybrids had six eigenvalues $> 1$, and the first axis accounted for 36.88% of the variation in the color of these fishes. The relatively lower number of eigenvalues with values $> 1$, and the greater percentage of variation explained by the first principal component, are both indicative of integration (Table 4).

Male–male aggression

All 10 focal male *L. trewavasae* responded to the stimulus males, yielding usable data for these analyses. The data were not normally distributed, so Wilcoxon signed rank tests were used to test for pairwise differences in the aggression directed to the two different opponents. The focal males did not discriminate between *L. trewavasae* and *L. fuelleborni*-sired hybrid opponents, directing approximately equal numbers of aggressive behaviors to either opponent. None of the differences were significant, neither the total sum of all aggressive behaviors (total aggression: $Z = -1.020, P = 0.308$; Fig. 6a), nor the number of individual aggressive behaviors (lateral displays: $Z = -0.803, P = 0.407$; frontal displays: $Z = -1.219, P = 0.223$; bites: $Z = -0.840, P = 0.401$; butts: $Z = -0.867, P = 0.386$).

---

Fig. 4 Reflectances obtained at the six body locations for all four treatment groups. The titles of each plot are the body region from which the reflectances were measured. Each curve is the average of all the members of a given treatment group; error bars are omitted for clarity. Yellow: *L. fuelleborni* ($n = 12$); blue: *L. trewavasae* ($n = 12$); green: *L. trewavasae*-sired hybrids ($n = 12$); red: *L. fuelleborni*-sired hybrids ($n = 7$)
Female mate choice

Of the 10 attempted trials, nine yielded usable data; in one case, a male was able to get around the permanent transparent divider and was found in direct contact with the female during the acclimation period. In the nine trials that provided usable data, the data were normally distributed, so parametric analyses were used to analyze the data.

With respect to the males, despite the difference in mean length between *L. trewavasae* and *L. fuelleborni*-sired hybrid males noted above, the difference in length between a pair of males did not influence the number of responses directed to either opponent ($R^2 = 0.124$; $F_{1,7} = 0.989$; $P = 0.353$). While the *L. fuelleborni*-sired hybrid males tended to perform more displays than *L. trewavasae* males (hybrids: $9.0 \pm 3.7$ displays; *L. trewavasae*: $3.6 \pm 1.1$ displays), this difference was not significant ($t_8 = 1.623$, $P = 0.143$). The great difference in the average number of displays performed was due to a single hybrid male who performed 34 displays, and, when this

| Variable                     | Caudal Peduncle PC 1 | Caudal Peduncle PC 2 | Dorsum PC 1 | Dorsum PC 2 | Dorsal Fin PC 1 | Dorsal Fin PC 2 |
|------------------------------|-----------------------|-----------------------|-------------|-------------|-----------------|-----------------|
| Total brightness             | 0.642                 | 0.463                 | 0.411       | 0.667       | 0.523           | 0.059           |
| Mean brightness              | 0.642                 | 0.463                 | 0.411       | 0.667       | 0.523           | 0.059           |
| Intensity                    | 0.138                 | 0.656                 | 0.439       | 0.651       | 0.467           | −0.004          |
| Ultraviolet chroma           | −0.491                | 0.404                 | 0.324       | −0.723      | 0.102           | −0.064          |
| Violet chroma                | −0.441                | 0.385                 | 0.372       | −0.670      | 0.192           | −0.009          |
| Blue chroma                  | 0.677                 | −0.245                | 0.162       | 0.674       | 0.286           | 0.406           |
| Green chroma                 | 0.374                 | −0.369                | −0.346      | 0.569       | −0.260          | −0.110          |
| Yellow chroma                | 0.289                 | −0.398                | −0.484      | 0.298       | −0.304          | −0.226          |
| Red chroma                   | 0.161                 | −0.407                | −0.387      | −0.272      | −0.279          | −0.314          |
| Spectral saturation          | −0.418                | 0.397                 | 0.317       | −0.154      | −0.057          | −0.322          |
| Contrast                     | −0.043                | 0.622                 | 0.473       | 0.576       | 0.221           | −0.091          |
| Peak $\lambda$               | 0.004                 | 0.016                 | −0.107      | 0.672       | −0.223          | 0.166           |
| $\lambda$ of maximum negative slope | 0.285                 | −0.193                | −0.361      | 0.163       | −0.364          | 0.215           |
| $\lambda$ of median reflectance | 0.193                 | −0.059                | 0.375       | −0.189      | 0.066           | −0.064          |

| Forehead                     |                      |                      |             |             |                 |                 |
|------------------------------|-----------------------|-----------------------|-------------|-------------|-----------------|-----------------|
| Total brightness             | 0.266                 | 0.724                 | 0.522       | 0.603       | 0.743           | 0.312           |
| Mean brightness              | 0.266                 | 0.724                 | 0.522       | 0.603       | 0.743           | 0.312           |
| Intensity                    | 0.353                 | 0.678                 | 0.703       | 0.376       | 0.630           | 0.447           |
| Ultraviolet chroma           | 0.544                 | −0.579                | 0.452       | −0.646      | −0.609          | 0.000           |
| Violet chroma                | 0.631                 | −0.498                | 0.517       | −0.615      | −0.504          | −0.149          |
| Blue chroma                  | 0.320                 | 0.537                 | 0.093       | 0.388       | 0.686           | −0.336          |
| Green chroma                 | −0.552                | 0.516                 | −0.489      | 0.594       | 0.461           | 0.287           |
| Yellow chroma                | −0.806                | 0.300                 | −0.495      | 0.541       | 0.425           | 0.289           |
| Red chroma                   | −0.800                | −0.129                | −0.564      | 0.393       | 0.096           | 0.230           |
| Spectral saturation          | 0.444                 | −0.309                | 0.402       | −0.560      | −0.600          | 0.036           |
| Contrast                     | 0.511                 | 0.519                 | 0.764       | 0.166       | 0.379           | 0.367           |
| Peak $\lambda$               | −0.423                | 0.332                 | −0.017      | 0.179       | 0.319           | 0.054           |
| $\lambda$ of maximum negative slope | −0.113                | −0.020                | −0.540      | 0.410       | −0.270          | −0.024          |
| $\lambda$ of median reflectance | −0.403                | −0.192                | −0.433      | 0.186       | 0.038           | −0.052          |
The findings of Pauers et al. (2018). Conversely, the parental species, and a coloration most similar to that of the males of the maternal species; this agrees with L. fuelleborni have a body shape intermediate to the L. trewavasae and a female L. trewavasae present a combination of morphological and color features that are unique and different to that of either parental species, as well as that of the reciprocal hybrid. As such, while the L. trewavasae-sired hybrids are phenotypically intermediate to their parental species, the L. fuelleborni-sired hybrids have a novel combination of morphometry and pigmentation, and are consistent with what would be expected in a transgressive hybrid.

Pauers et al. (2018) found that the body shape of F1 hybrids created by crossing female L. fuelleborni and male L. trewavasae contributed to a reduced foraging efficiency. In this study, hybrids produced by L. trewavasae sires and L. fuelleborni dams had an upturned and expanded neurocranium not found in either of the parental species that contributed a slower growth rate in laboratory conditions (Pauers et al., 2018). Given how consequential body shape is for foraging and maneuvering in the aquatic environment (Higham et al., 2015), especially for the algae-grazing cichlids of Lake Malawi (Stauffer & Posner, 2006; Rupp and Hulsey, 2014), the inability for these hybrids to thrive under these conditions is not surprising. It would thus be especially enlightening to see how the L. fuelleborni-sired hybrids fare in a similar experiment.

Brzozowski et al. (2014) and Albertson et al. (2014), using their innovative approach to the analysis of color patterns of the cichlids of Lake Malawi, found that males have less integrated, more modular color patterns. Such color patterns, consisting of separate, distinct units of pigmentation, would allow them to evolve rapidly, especially in response to sexually selection (Brzozowski et al., 2014; Albertson et al., 2014). In their analyses, they used the number of eigenvalues > 1, as well as the amount of variance explained by each principal component, to determine the degree to which color patterns were modular; a large number of eigenvalues > 1 and a lower amount of variance explained by each principal component indicated a greater degree of modularity, versus integration, in a color pattern. Our results of the principal components analysis of the spectral data are consistent with this pattern. Male L. fuelleborni, L. trewavasae, and L. trewavasae-sired hybrids, while differing somewhat in their spectral characteristics, nonetheless have modular color patterns, as demonstrated by the relatively large number of eigenvalues > 1, and the
lower amount of variance explained by each principal component axis. Conversely, the *L. fueleborni*-sired hybrid males had a spectrally unique color pattern, one that was rich in ultraviolet wavelengths, but lacking violet and blue wavelengths, at several of the body regions we sampled. Additionally, for this treatment group, there were fewer eigenvalues > 1, and a greater percentage of variance was explained by each principal component.

It is not surprising, then, that these transgressive hybrid males would not be recognized by female *L. trewavasae* as a potential mate. Female rock-dwelling cichlids of Lake Malawi, and especially the *Labeotropheus*, have long been known to select their mates visually, especially on the basis of male nuptial color (Jordan et al., 2003; Knight & Turner, 2004; Pauers et al., 2004; Pauers & Grudnowski, 2022). The body shape of these *L. fueleborni*-sired hybrids, and especially their unusual coloration, makes them visually distinct from *L. trewavasae* males, at least to a human observer, although the spectral distribution of their color pattern also suggests that these differences should be

| Table 3 Analysis of variance for color principal components |
|-----------------------------------------------------------|
| (A) Color PC 1 *(n = 38; multiple R² = 0.584)*          |
| Variable                                                 | Sum of squares | df | Mean square | F    | P       |
| Treatment Group                                          | 21.619         | 3  | 7.206       | 15.929 | ≤ 0.001 |
| Error                                                   | 15.381         | 34 | 0.452       |       |         |
| Pairwise comparisons                                     |                |    |             |       |         |
| *L. fueleborni*                                          | Hybrid 1 (*Lt Sire*) | Hybrid 2 (*Lf Sire*) |
| Hybrid 1 (*Lt Sire*)                                     | − 0.288***     |     |            |       |         |
| Hybrid 2 (*Lf Sire*)                                     | 1.080*         | 1.368*** |         |       |         |
| *L. trewavasae*                                          | 1.183**        | 0.895* | 2.263*** |       |         |

*ns not significant
*P = 0.05; **P = 0.01; ***P = 0.001

| Table 4 Number of eigenvalues and percentage of variance explained by each principal component for the principal component analyses performed on the spectral data for each treatment group |
|---------------------------------------------------------------|
| Treatment group                                             | Number of eigenvalues > 1 | Percent variance explained by each axis |
|                                                              |                             | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  |
| *L. fueleborni*                                              | 9                            |     | 29.81 | 16.12 | 13.90 | 11.30 | 8.78 | 6.84 | 5.44 | 4.19 | 3.62 | –   |
| *L. trewavasae*                                              | 9                            |     | 30.20 | 17.86 | 14.31 | 11.74 | 8.62 | 6.71 | 4.37 | 3.93 | 2.26 | –   |
| *L. fueleborni*-sired hybrids                                | 6                            |     | 36.88 | 18.51 | 17.40 | 12.74 | 9.83 | 4.64 | –   | –   | –   | –   |
| *L. trewavasae*-sired hybrids                                | 10                           |     | 29.73 | 18.25 | 12.44 | 10.29 | 9.72 | 5.79 | 5.13 | 4.00 | 3.18 | 1.46 |

sired hybrid males had a spectrally unique color pattern, one that was rich in ultraviolet wavelengths, but lacking violet and blue wavelengths, at several of the body regions we sampled. Additionally, for this treatment group, there were fewer eigenvalues > 1, and a greater percentage of variance was explained by each principal component.
visible to female *L. trewavasae* (Carleton & Kocher, 2001; Pauers et al., 2004).

The results of the male-male aggression experiments are less clearly interpreted. Male rock-dwelling cichlids from Lake Malawi use the color patterns of their opponents to ascertain the greatest threat to their ability to attract a mate (i.e., males colored most similarly to themselves) and modulate their aggression accordingly (Pauers et al., 2008; Pauers & McKinnon, 2012; Pauers & Grudnowski, 2020). Given the differences in coloration between *L. trewavasae* and *L. fuelleborni*-sired males, it is reasonable to expect that focal male *L. trewavasae* would direct more aggressive behaviors to *L. trewavasae* opponents, but this was not the case. The focal male *L. trewavasae* in these experiments directed equal numbers of aggressive behaviors to both opponents. It could be possible that the spectral characteristics of the color pattern of *L. fuelleborni*-sired hybrids are just similar enough to those of *L. trewavasae* that they do not meet some inherent threshold of species recognition (Crapon de Caprona, 1986; Ribbink, 1991), assuming that males identify their opponents using visual cues (Pauers et al., 2008, Pauers & McKinnon, 2012). The results from this experiment suggest that, in a natural and unaltered habitat, the *L. fuelleborni*-sired hybrid males would be treated as a conspecific by males of both parental species, and would thus have no advantage over male *L. trewavasae* during intrasexual aggressive encounters. To the best of our knowledge, this is the first time that the performance of male hybrids of Lake Malawi cichlids in intrasexual aggressive encounters has been tested.

Taken together, the results of our behavioral experiments strongly suggest that these transgressive *L. fuelleborni*-sired hybrids are not likely to be successful competitors or mates in a natural population in an undisturbed habitat (but see Seehausen et al., 1997). Young rock-dwelling cichlids in Lake Malawi typically do not migrate more than a few meters from where they are released, and are thus most likely to join the community frequented by their mothers (Ribbink et al., 1983; Ribbink, 1991). Since the *L. fuelleborni*-sired hybrid males used in our experiments were carried by female *L. trewavasae*, in Lake Malawi they would come to sexual maturity surrounded by *L. trewavasae*; our results suggest that these hybrids would struggle in sexually selected interactions with adults of their parental species.

Given the lack of *L. fuelleborni* females and *L. trewavasae*-sired hybrid males in our experiments, results of our behavioral studies provide an incomplete picture of the way hybrid males might interact in a natural hybrid zone of *Labeotropheus*. Nonetheless, these results, coupled with the morphological and spectrophotometric data, allow us to propose further investigations to determine whether or not these findings are broadly applicable. An obvious place to start would be to perform reciprocal experiments in which female *L. fuelleborni* are offered a choice between *L. trewavasae*-sired hybrid males and male *L. fuelleborni*. Since the *L. trewavasae*-sired hybrid males are very similar in appearance to *L. fuelleborni*, both morphologically and chromatically, it might be possible that these hybrids would receive attention from female *L. fuelleborni*. With regards to male-male aggression, the similarity, at
least to a human observer, between *L. trewavasae*-sired and *L. fueleborni* males suggests that focal male *L. fueleborni* should direct equal amounts of aggression towards conspecific and *L. trewavasae*-sired opponents. Finally, it would be extremely informative to examine the mate choice preferences of both *L. fueleborni* - and *L. trewavasae*-sired females. If these females prefer non-hybrid, parental males, that could explain the presence and persistence of gene flow among the cichlids of Lake Malawi (Mims et al., 2010; Selz et al., 2014a, b; Hulsey et al., 2017). On the other hand, if hybrid females prefer hybrid males, this could lend credence to the hypothesis that hybridization is an important source of phenotypically divergent species in Lake Malawi (Feller et al., 2020).

Although morphological and behavioral evidence from the wild is exceedingly rare, molecular genetics reveals that hybridization has undoubtedly occurred during the evolution of the species flock of cichlids endemic to Lake Malawi (e.g., Mims et al., 2010; Loh et al., 2013; Meier et al., 2017; Malinsky et al., 2018; Svardal et al., 2020). Our results indicate that hybridization does indeed generate phenotypic novelty in the *Labeotropheus*, but that the emergence of novelty depends upon the parentage of the hybrids, since one direction produces intermediate hybrids, and the other produces novel combinations of traits. Perhaps most importantly, our experiments suggest that one reason for the rarity of hybrids in modern day Lake Malawi could be the fact that transgressive males were neither preferred by females of their maternal species, nor did they receive less aggression from males of the maternal species.

In the vast majority of studies involving hybridization in cichlids from Lake Malawi, experimental crosses were made in only a single direction (e.g., McElroy & Kornfield, 1993; Albertson & Kocher, 2001; Brzozowski et al., 2014; Pauers et al., 2018). In the relatively few studies in which reciprocal crosses were made, the resulting hybrid progeny were pooled in the subsequent analyses, thus obscuring their parentage (e.g., Husemann et al., 2017). Given the results presented in this paper, as well as the roles sexual conflict and chromosomal sex determination play in determining the chromatic phenotype of male cichlids from Lake Malawi (Roberts et al., 2009), it is important for future studies to compare hybrid offspring from reciprocal crosses.

**Funding** This project was supported by the Orth Family Ichthyology Research Fund of the Milwaukee Public Museum, the American Cichlid Association Guy D. Jordan Research Fund, and by donations of aquarium equipment by Aqueon, Inc. (Franklin, Wisconsin, USA). LJA’s participation in these studies was made possible by a UW-Milwaukee Supporting Undergraduate Research Fellowship. The experiments summarized in this paper adhered to the ASAB/ABS Guidelines for the Use of Animals in Research, the legal requirements of the United States of America and the guidelines of the University of Wisconsin-Milwaukee Institutional Animal Care and Use Committee. This research project was reviewed and approved by the UWM IACUC and assigned animal care and use Protocol number 18-19#10.

**Data availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Competing Interests** None of the authors have any competing interests to report.

**References**

Albertson, R. C. & T. D. Kocher, 2001. Assessing morphological differences in an adaptive trait: a landmark-based morphometric approach. *Journal of Experimental Zoology* 289: 385–403.

Albertson, R. C., K. E. Powder, Y. Hu, K. P. Coyle, R. B. Roberts & K. J. Parsons, 2014. Genetic basis of continuous variation in the levels and modular inheritance of pigmentation in cichlid fishes. *Molecular Ecology* 23: 5135–5150.

Barlow, G. W., 1993. The puzzling paucity of feeding territories among freshwater fishes. *Marine Behaviour and Physiology* 23: 155–174.

Barlow, G. W., 2000. The Cichlid Fishes: Nature’s Grand Experiment in Evolution. Perseus Publishing, Cambridge.

Barlow, G. W., 2002. How behavioural studies contribute to the species problem: a piscine perspective. *Fish and Fisheries* 3: 197–202.

Brzozowski, F., J. Roscoe, K. Parsons & R. C. Albertson, 2014. Sexually dimorphic levels of color trait integration and the resolution of sexual conflict in Lake Malawi cichlids. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* 318: 268–278.

Carleton, K. L. & T. D. Kocher, 2001. Cone opsins genes of African cichlid fishes: tuning spectral sensitivity by differential gene expression. *Molecular Biology and Evolution* 18: 1540–1550.

Concannon, M. R. & R. C. Albertson, 2015. The genetic and developmental basis of an exaggerated craniofacial trait in East African cichlids. *Journal of Experimental Zoology B: Molecular Development and Evolution* 324: 662–670.
Couldridge, V. C. K. & G. J. Alexander, 2001. Does the time spent near a male predict female mate choice in a Malawian cichlid? Journal of Fish Biology 59: 667–672.

Coyne, J. A. & H. A. Orr, 2004. Speciation. Sinauer Associates, Sunderland.

Crapon de Caprona, M.-D., 1986. The use of fertile hybrids for the study of the accuracy of species recognition in cichlids. Annals of the Royal Museum for Central Africa 251: 117–120.

Danley, P. D., M. Husemann, B. Ding, L. M. Dipietro, E. J. Beverly & D. J. Peppe, 2012. The impact of the geologic history and paleoclimate on the diversification of East African cichlids. International Journal of Evolutionary Biology 2012: 574851.

Egger, B., K. M. Sefc, L. Makasa, C. Sturmbauer & W. Sturmbauer, 2012. Intergeneric hybridization between color morphs in a population cichlid fishes twelve years after human-induced secondary admixture. Journal of Heredity 103: 515–522.

Feller, A. F., O. M. Selz, M. D. McGee, J. I. Meier, S. Mwaiko & O. Seehausen, 2020. Rapid generation of ecologically relevant behavioral novelty in experimental cichlid hybrids. Ecology and Evolution 2020: 7445–7462.

Gante, H. F., M. Matschiner, M. Jakobsen, S. Jenoft & W. Salzburger, 2016. Genomics of speciation and introgression in Princess cichlid fishes from Lake Tanganyika. Molecular Ecology 25: 6143–6161.

Genner, M. J. & G. F. Turner, 2012. Ancient hybridization and phenotypic novelty within Lake Malawi’s cichlid fish radiation. Molecular Biology and Evolution 29: 195–206.

Genner, M. J. & G. F. Turner, 2015. Timing of population expansions within the Lake Malawi haplochromine cichlid fish radiation. Hydrobiologia 748: 121–132.

Haesler, M. P. & O. Seehausen, 2005. Inheritance of female mating preference in a sympatric sibling species pair of Lake Victoria cichlids: implications for speciation. Proceedings of the Royal Society of London Series B 272: 237–245.

Hedrick, P. W., 2013. Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. Molecular Ecology 22: 4606–4618.

Higham, T. E., W. J. Stewart & P. C. Wainwright, 2015. Turbulence, temperature, and turbidity: the ecomechanics of predator-prey interactions in fishes. Integrative and Comparative Biology 55: 6–20.

Holzman, R. & C. D. Hulse, 2017. Mechanical transgressive segregation and the rapid origin of trophic novelty. Scientific Reports 7: 40306.

Husemann, M., M. Tobler, C. McCauley, B. Ding & P. D. Danley, 2017. Body shape differences in a pair of closely related Malawi cichlids and their hybrids: effects of genetic variation, phenotypic plasticity, and transgressive segregation. Ecology and Evolution 7: 4336–4346.

Hulse, C.D., J. Zheng, B.C. Faircloth, A. Meyer & M.E. Alfaro, 2017. Phylogenomic analysis of Lake Malawi cichlid fishes: Further evidence that the three-stage model of diversification does not fit. Molecular Phylogenetics and Evolution 114: 40–48.

Ivory, S. J., M. W. Blome, J. W. King, M. M. McGlue, J. E. Cole & A. S. Cohen, 2016. Environmental change explains cichlid adaptive radiation at Lake Malawi over the past 1.2 million years. Proceedings of the National Academy of Sciences USA 113: 11895–11900.

Jordan, R., K. Kellogg, F. Juanes & J. R. Stauffer Jr., 2003. Evaluation of female mate choice cues in a group of Lake Malawi Mbuna (Cichlidae). Copeia 2003: 181–186.

Klingenberg, C. P., 2009. Morphometric integration and modularity in configurations of landmarks: tools for evaluating a priori hypotheses. Evolution and Development 11: 405–421.

Knight, M. E. & G. F. Turner, 2004. Laboratory mating trials indicate incipient speciation by sexual selection among populations of the cichlid fish Pseudotropheus zebra from Lake Malawi. Proceedings of the Royal Society B 271: 675–680.

Koblmüller, S., B. Nevado, L. Makasa, M. Van Steenberge, M. P. M. Vanhove, E. Verheyen & C. K. M. SturmbauerSefc, 2017. Phylogeny and phylogeography of Altolamprologus: ancient introgression and recent divergence in a rock-dwelling Lake Tanganyika cichlid genus. Hydrobiologia 791: 35–50.

Kornfield, I. L. & P. F. Smith, 2000. African cichlid fishes: model systems for evolutionary biology. Annual Review of Ecology and Systematics 31: 163–196.

Loh, Y.-H.E., E. Bezault, F. M. Muenzel, R. B. Roberts, R. Swofford, M. Barluenga, C. E. Kidd, A. E. Howe, F. Di Palma, K. Lindblad-Toh, J. Hey, O. Seehausen, W. Salzburger, T. D. Kocher & J. T. Steelman, 2013. Origins of shared genetic variation in African cichlids. Molecular Biology and Evolution 30: 906–917.

Maia, R., H. Gruson, J. A. Endler & T. E. White, 2019. pavo 2: new tools for the spectral and spatial analysis of colour in R. Methods in Ecology and Evolution 2019: 1097–1107.

Malinsky, M., H. Svardal, A. M. Tyers, E. A. Miska, M. J. Genner, G. F. Turner & R. Durbin, 2018. Whole-genome sequences of Malawi cichlids reveal multiple radiations interconnected by gene flow. Nature Ecology and Evolution 2: 1940–1955.

Mayr, E., 1963. Animal Species and Evolution. Belknap Press, Cambridge.

McElroy, D. M. & I. Kornfield, 1993. Novel jaw morphology in hybrids between Pseudotropheus zebra and Labeotropheus fuelleborni (Teleostei: Cichlidae) from Lake Malawi, Africa. Copeia 1993: 933–945.

Meier, J. I., D. A. Marques, S. Mwaiko, C. E. Wagner, L. Excoffier & O. Seehausen, 2017. Ancient hybridization fuels rapid cichlid fish adaptive radiations. Nature Communications 8: 14363.

Meyer, B. S., M. Matschiner & W. Salzburger, 2017. Disentangling incomplete lineage sorting and introgression to refine species-tree estimates for Lake Tanganyika cichlid fishes. Systematic Biology 66: 531–550.

Mims, M. C., C. D. Hulse, B. M. Fitzpatrick & J. T. Steelman, 2010. Geography disentangles introgression from ancestral polymorphism in Lake Malawi cichlids. Molecular Ecology 19: 940–951.

Pauers, M. J. & J. A. Grudnowski, 2020. Assortative aggression among males in a sympatric pair of Labeotropheus
from Lake Malawi, Africa. Journal of Fish Biology 2020: 1–7.

Pauers, M. J. & J. A. Grudnowski, 2022. Female preferences for conspecific males indicate reproductive isolation between sympatric Labeotropheus Ahl from Lake Malawi. Ethology 128: 482–488.

Pauers, M. J. & J. S. McKinnon, 2012. Sexual selection on color and behavior within and between cichlid populations: implications for speciation. Current Zoology 58: 475–483.

Pauers, M. J. & S. A. McMillan, 2015. Geomorphic morphometrics reveals surprising diversity in the Lake Malawi cichlid genus Labeotropheus. Hydrobiologia 748: 145–160.

Pauers, M. J., J. S. McKinnon & T. J. Ehlinger, 2004. Directional sexual selection on chroma and within-pattern color contrast in Labeotropheus fuelleborni. Proceedings of the Royal Society of London Series B 271: S444–S447.

Pauers, M. J., J. M. Kapfer, C. E. Fendos & C. S. Berg, 2008. Aggressive biases towards similarly coloured males in Lake Malawi cichlid fishes. Biology Letters 4: 156–159.

Pauers, M. J., T. J. Ehlinger & J. S. McKinnon, 2010. Female and male visually based mate preferences are consistent with reproductive isolation between populations of the Lake Malawi endemic Labeotropheus fuelleborni. Current Zoology 56: 65–72.

Pauers, M. J., K. R. Fox, R. A. Hall & K. Patel, 2018. Selection, hybridization, and the evolution of morphology in the Lake Malawi endemic cichlids of the genus Labeotropheus. Scientific Reports 2018: 15842.

Ribbink, A. J., 1990. Alternative life-history styles of some African cichlid fishes. Environmental Biology of Fishes 28: 87–100.

Ribbink, A. J., 1991. Distribution and ecology of the cichlids of the African Great Lakes. In Keenleyside, M. H. A. (ed), Cichlid Fishes: Behaviour, Ecology and Evolution. Chapman & Hall, London: 36–59.

Ribbink, A. J., B. A. Marsh, A. C. Marsh, A. C. Ribbink & B. J. Sharp, 1983. A preliminary survey of the cichlid fishes of rocky habitats in Lake Malawi. South African Journal of Zoology 18: 149–310.

Roberts, R. B., J. R. Ser & T. D. Kocher, 2009. Sexual conflict resolved by invasion of a novel sex determinant in Lake Malawi cichlid fishes. Science 326: 998–1001.

Rohlf, F.J. 2021. tpsRelw: Relative warps analysis, version 1.75. https://www.sbmorphometrics.org/morphmet/tpsRelw64.exe.

Romesch, S. J., J. Torres-Dowdall, G. Machado-Schiaffino, N. Karagic & A. Meyer, 2021. Dual function and associated costs of a highly exaggerated trait in a cichlid fish. Ecology and Evolution 11: 17496–17508.

Rupp, M. F. & C. D. Hulsey, 2014. Influence of substrate orientation on feeding kinematics and performance of algae grazing Lake Malawi cichlid fishes. Journal of Experimental Biology 217: 3057–3066.

Santos-Santos, J.H., L. Audenaert, E. Verheyen & D. Adriaens, 2021. Ontogenetic divergence generates novel phenotypes in hybrid cichlids. Anatomy 238: 1116–1127.

Seehausen, O., J. J. M. van Alphen & F. Witte, 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. Science 277: 1808–1811.

Selz, O. M. & O. Seehausen, 2019. Interspecific hybridization can generate functional novelty in cichlid fish. Proceedings of the Royal Society of London Series B 286: 20191621.

Selz, O. M., R. Thommen, M. E. Mann & O. Seehausen, 2014a. Behavioural isolation may facilitate homoploid hybrid speciation in cichlid fish. Journal of Evolutionary Biology 27: 275–289.

Selz, O. M., K. Lucek, K. A. Young & O. Seehausen, 2014b. Relaxed trait covariance in interspecific cichlid hybrids predicts morphological diversity in adaptive radiations. Journal of Evolutionary Biology 27: 11–24.

Smith, P. F., A. Konings & I. Kornfield, 2003. Hybrid origin of a cichlid population in Lake Malawi: implications for genetic variation and species diversity. Molecular Ecology 12: 2497–2504.

Stauffer, J. R., Jr. & I. Posner, 2006. An investigation of the utility of feeding angles among Lake Malawi rock-dwelling cichlids (Teleostei: Cichlidae). Copeia 2006: 289–292.

Stauffer, J. R., Jr., N. J. Bowers, T. D. Kocher & K. R. McKay, 1996. Evidence of hybridization between Cynotilapia afra and Pseudotropheus zebra (Teleostei: Cichlidae) following an intralacustrine translocation in Lake Malawi. Copeia 1996: 203–208.

Stellens, R. B., C. Schmid, O. Selz & O. Seehausen, 2009. Phenotypic novelty in experimental hybrids is predicted by the genetic distance between species of cichlid fish. BMC Evolutionary Biology 9: 283.

Svardal, H., F. X. Quah, M. Malinsky, B. P. Ngatunga, A. E. Miska, W. Salzburger, M. J. Genner, G. F. Turner & R. Durbin, 2020. Ancestral hybridization facilitated species diversification in the Lake Malawi cichlid fish adaptive radiation. Molecular Biology and Evolution 37: 1100–1113.

Svensson, O., B. Egger, B. Gricar, K. Woodhouse, C. van Oosterhout, W. Salzburger, O. Seehausen & G. F. Turner, 2011. Segregation of species-specific male attractiveness in F2 hybrid Lake Malawi cichlid fish. International Journal of Evolutionary Biology 2011: 1–7. https://doi.org/10.4061/2011/426179.

Svensson, O., K. Woodhouse, C. van Oosterhout, A. Smith, G. F. Turner & O. Seehausen, 2017. The genetics of mate preferences in hybrids between two young and sympatric Lake Victoria cichlid species. Proceedings of the Royal Society of London Series B 284: 20162332.

van Snick Grey, E. & J. R. Stauffer Jr., 2004. Phenotypic plasticity: its role in trophic radiation and explosive speciation in cichlids (Teleostei: Cichlidae). Animal Biology 54: 137–158.

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.