



Female mating preferences and male coloration covary with water transparency in a Lake Victoria cichlid fish

MARTINE E. MAAN^{1,2,3*}, OLE SEEHAUSEN^{2,3} and JACQUES J. M. VAN ALPHEN^{1,4}

¹Department of Animal Ecology, Institute of Biology, University of Leiden, PO Box 9516, 2300 RA Leiden, the Netherlands

²Department of Fish Ecology and Evolution, EAWAG Swiss Federal Institute of Aquatic Science and Technology, Center of Ecology, Evolution and Biochemistry, Seestrasse 79, CH-6047 Kastanienbaum, Switzerland

³Department of Aquatic Ecology and Macroevolution, Institute of Ecology and Evolution, University of Bern, Baltzerstrasse 6, CH-3012 Bern, Switzerland

⁴Université de Rennes 1, UMR 6553 Ecobio, Avenue du Général Leclerc, Campus de Beaulieu, 35042 Rennes, France

Received 19 July 2009; accepted for publication 9 September 2009

Rapid speciation in Lake Victoria cichlid fish of the genus *Pundamilia* may be facilitated by sexual selection: female mate choice exerts sexual selection on male nuptial coloration within species and maintains reproductive isolation between species. However, declining water transparency coincides with increasingly dull coloration and increasing hybridization. In the present study, we investigated the mechanism underlying this pattern in *Pundamilia nyererei*, a species that interbreeds with a sister species in turbid but not in clear water. We compared measures of intraspecific sexual selection between two populations from locations that differ in water transparency. First, in laboratory mate-choice experiments, conducted in clear water and under broad-spectrum illumination, we found that females originating from turbid water have significantly weaker preferences for male coloration than females originating from clear water. Second, both the hue and body coverage of male coloration differ between populations, which is consistent with adaptation to different photic habitats. These findings suggest that the observed relationship between male coloration and water transparency is not mediated by environmental variation alone. Rather, female mating preferences are indicated to have changed in response to this variation, constituting the first evidence for intraspecific preference-trait co-evolution in cichlid fish. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, **99**, 398–406.

ADDITIONAL KEYWORDS: divergence – eutrophication – haplochromine – nuptial – *Pundamilia nyererei* – reproductive isolation – sexual selection – speciation.

INTRODUCTION

Animals adjust their sexual signals to environmental conditions. Differential employment or elaboration of visual, acoustic, seismic or behavioural displays has been demonstrated both at the intraspecific (spiders: Taylor & Jackson, 1999) and interspecific level (birds: Badyaev, Hill & Weckworth, 2002). Furthermore, human-induced changes in the

environment may influence the nature and efficacy of animal signals (Seehausen, Van Alphen & Witte, 1997; Slabbekoorn & Peet, 2003; Fisher, Wong & Rosenthal, 2006).

In water, visual communication is constrained by the light transmission properties of the water and its organic and inorganic content (Lythgoe, 1984). Water turbidity can severely hamper sexual selection on visual cues (Candolin, Salesto & Evers, 2007). Furthermore, the wavelength distribution of the transmitted light determines the colour of the background against which a signal is viewed, influencing

*Corresponding author. E-mail: martine@martinemaan.nl

its conspicuousness (Marshall, 2000; Fuller, 2002). The ambient spectrum also determines the 'colour space' available for reflection and perception (Endler, 1990; Seehausen, Van Alphen & Witte, 1997). In guppies and sticklebacks, male colour traits and female preferences for these traits co-vary across populations that differ in water colour (Endler & Houde, 1995; Boughman, 2001).

Water transparency in Lake Victoria has been declining for several decades (Verschuren *et al.*, 2002). Because many haplochromine cichlids use visual cues in mate choice (Seehausen & Van Alphen, 1998; Knight & Turner, 2004; Pauers, McKinnon & Ehlinger, 2004), increasing turbidity poses a threat to species diversity (Seehausen *et al.*, 1997). *Pundamilia pundamilia* and *Pundamilia nyererei* are two closely-related species that co-occur at many islands in Lake Victoria. Females of both species are yellowish brown, but male *P. pundamilia* are metallic blue and male *P. nyererei* are bright red and yellow. Gene flow between the species is restricted by female mating preferences for different male nuptial coloration (Seehausen & Van Alphen, 1998), although the two phenotypes hybridize in turbid locations.

Recent work indicates that water transparency covaries with the extent of depth segregation, differentiation in the visual system, and the strength of assortative mating between these species at different islands (Seehausen *et al.*, 2008). However, even between islands where the two species are strongly differentiated, male coloration covaries with water turbidity: males of *P. nyererei* become less red with decreasing water transparency, and this variation is heritable (Seehausen *et al.*, 1997; Seehausen *et al.*, 2008). The underlying evolutionary mechanism is unknown. Previous work has shown that, in a clear water population of *P. nyererei*, female choice exerts directional sexual selection on male red coloration (Maan *et al.*, 2004). In turbid water, such selection may be weaker. This may happen because female preferences evolve in response to variation in water transparency, resulting in weaker selection on male red coloration in turbid waters. Alternatively, the strength of selection on male coloration by female choice may be a direct function of signal transduction through the water, without mating preferences evolving themselves.

In the present study, we investigate these alternatives. We document mate choice behaviour of a *P. nyererei* population from turbid water and compare this with the previously established preference behaviour of a population from clear water (Maan *et al.*, 2004). Both populations are tested under identical laboratory conditions. We also quantify male coloration and the light environment in both populations.

MATERIAL AND METHODS

FISH

Male *Pundamilia nyererei* are bright red dorsally and have yellow sides intercepted by black vertical bars. Females are yellowish brown with dark vertical bars. We compared *P. nyererei* populations from two islands in south-eastern Lake Victoria that differ in water transparency: Kissenda (turbid water; Secchi disk reading mean \pm SE = 67.5 \pm 1.4 cm, $N = 4$, in the study period) and Makobe (clear water; 221 \pm 15 cm; $N = 29$; Fig. 1). Hybrids between *P. nyererei* and *P. pundamilia* have not been observed at Makobe Island, but they occur in low frequencies at Kissenda: in 2001–03, we caught one male of hybrid phenotype with every 15 males of *P. nyererei* phenotype ($N = 79$ fish). Genetic differentiation between blue and red phenotypes is significant at both islands, although it is greater at Makobe (microsatellite $F_{ST} = 0.026$) than at Kissenda (microsatellite $F_{ST} = 0.010$; Seehausen *et al.*, 2008). At Makobe, *P. nyererei* territories are most abundant at 4–7 m in depth and fish were collected by gillnetting. At Kissenda, *P. nyererei* predominantly occur at 2–5 m depth and fish were collected by gill-

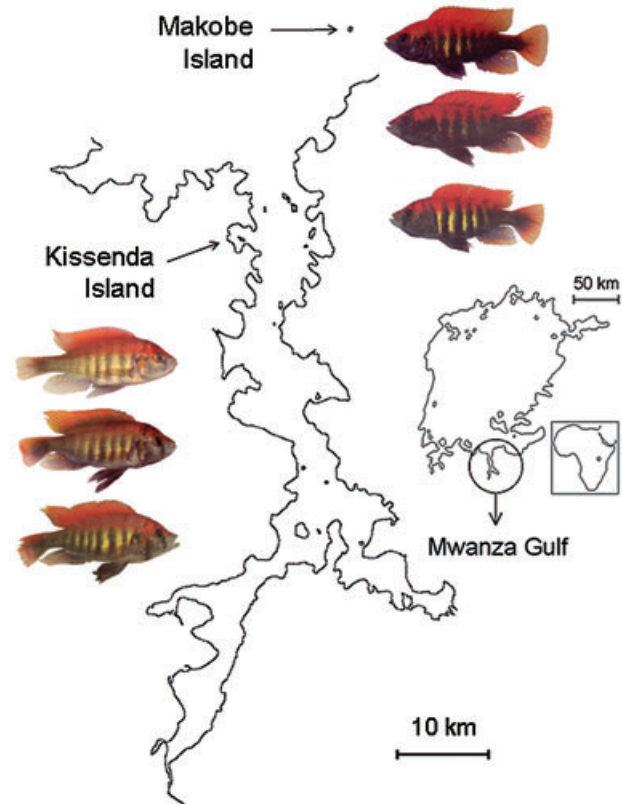


Figure 1. Study area and representative examples of *Pundamilia nyererei* males from Kissenda and Makobe Islands.

netting and angling. In both populations, fish were collected between December 2000 and April 2001 and transported to Leiden University (the Netherlands). Fish were kept in single-population groups in 250-L aquaria with continuous water flow, at 24–26 °C and under a 12 : 12 h light/dark cycle. Fish were fed commercial pellets (twice a week) and a mixture of shrimps and green peas (four times a week). A microchip was inserted into the abdominal cavity of each individual for identification (12 mm glass tags, type UKID122GL; Biomark Inc.). In the lake, *P. nyererei* males defend territories on the rocky bottom and attract females by vigorous courtship (Seehausen & Van Alphen, 1998). Courtship typically starts with Lateral Display, in which the male positions itself perpendicular to the female and spreads all fins. This is followed by Quiver, a high-frequency shaking movement of the body. Finally, the male leads the female to the centre of the territory in a quick swimming bout, often with exaggerated tail beats (Lead Swim). Spawning typically occurs in rocky crevices. The female lays one to four eggs at once, and immediately turns to pick them up when the male releases sperm. This sequence is repeated several times, with the pair swimming in circles around each other. After spawning, females mouthbrood eggs and larvae for approximately 3 weeks and guard the fry for an additional week after releasing them (Seehausen, 1996).

MATE CHOICE EXPERIMENTS

Mate-choice experiments were carried out in the laboratory, between November 2001 and August 2002, in accordance with the methods of Maan *et al.* (2004). Both populations were tested under identical conditions, in clear water, and under broad-spectrum illumination (40 W daylight fluorescent tubes). Each male was kept in one of two six-sided perspex enclosures (140 L) on either side of an 1800-L aquarium. In each enclosure, shelter was provided by a brick on top of a polyvinyl chloride (PVC) tube. These shelters were readily accepted by the males as the centres of their territories. In the main tank, two large stones provided shelter for the test female and blocked visual contact between males. The water in the male enclosures was filtered internally, preventing chemical communication. Air stones were present in both enclosures and in the main tank; water temperature was maintained at 24–26 °C.

Preceding experiments, males were photographed and measured (standard length), and assembled into male pairs of fixed composition. Males were paired such that we obtained a range of differences in red coloration, at the same time as reducing differences in body size (to less than 7% of the mean size of the two males in a pair).

For trials with Makobe females (from clear water), we used 11 females and seven male pairs. Redscore differences between paired males varied in the range 1–30% body coverage (for colour scoring, see below). For trials with Kissenda females (from turbid water), we used nine females and five male pairs with redscore differences in the range 3–12% body coverage. To add variation in redscore differences in the Kissenda trials, we assembled two additional male pairs using Makobe males, with redscore differences of 9% and 24.8% body coverage (these males were not used in the trials with Makobe females).

Males were released into their compartments the evening before trials. A maximum of three females was tested with one male pair in a day and consecutive trials were conducted at least 2 h apart. Male pairs were exchanged after three successful trials or after 2 days. The position of the males (left or right) was reversed each time the pair was introduced. To start a trial, the test female was released in the middle of the tank. Observation time started when the test female was within 30 cm of either one of the male compartments and stopped when she left this area. This distance was chosen because, in field observations, any fish within 30 cm of a *P. nyererei* territory elicits a behavioural response of the territory owner, indicating 30 cm as comprising an interaction threshold distance. This distance was also used in earlier investigations (Seehausen & Van Alphen, 1998; Maan *et al.*, 2004). Behaviour was recorded with OBSERVER, 3.0 (Noldus Information Technology). Trials were completed once 15 min of observation time had been collected.

For data analysis, we used only trials in which both males courted (performing Quiver or Lead Swim at least once) and the test female approached at least one of them. This yielded 48 trials for Makobe females and 43 trials for Kissenda females, in which each male pair was tested with four to nine females (mean \pm SE = 6.4 \pm 0.4).

MALE COLORATION AT MAKOBE AND KISSENDA ISLANDS

To quantify male colour variation in the field, we collected an additional sample of adult males from both islands. At Makobe, we collected 28 territorial and sexually active males, as established during scuba observations. Water transparency at Kissenda does not permit underwater observations. Therefore, we collected 17 males that expressed red, yellow, and black nuptial coloration and that belonged to the largest 10% of the sampled male population. Immediately after capture, males were photographed for colour analysis and sacrificed on melting ice. They

were subsequently measured (standard length, to the nearest 0.1 mm) and weighed (to the nearest 0.1 g). For photography, males were placed in a perspex cuvette with water and gently squeezed against the front window of the cuvette with a grey PVC sheet in the background. We used an SLR camera and two flashes on either side and subsequently digitized pictures. We adjusted white balance in PhotoShop 6.0 (Adobe Systems Inc.) using a white patch (Kodak colour card) attached to the front of the cuvette. We calculated colour scores in SIGMASCAN PRO, version 4.0 (SPSS Inc.). *Sensu* Maan *et al.* (2004), we defined criteria delimiting the body area (excluding fins and eyes) covered by red and yellow by a combination of hue (RGB) and saturation. RGB hue is in the range 0–255, where 0 indicates red, 40 yellow, 90 green, 170 blue, 210 purple, and 255 red again. Saturation ranges from 0 (grey) to 100 (full colour). We defined red as hue = 0–26 plus 232–255, and yellow : hue = 27–45. Saturation limits were 40–97. We similarly defined criteria for blackness: intensity = 0–75 [intensity ranges from 0 (black) to 255 (white)]. By calculating the area of the fish body that matched these criteria, we obtained a percentage of body coverage, subsequently referred to as redscore, yellowscore, and blackscore. To compare male coloration between populations, we also calculated the average hue of the male dorsum (dorsal hue), measured using the colour sampler tool in Adobe PhotoShop 6.0 (Adobe Systems Inc.) in five standardized positions (forehead, dorsal fin attachment point, anterior dorsum, central dorsum, posterior dorsum). Hue was defined as a location on the standard colour wheel, expressed as a degree in the range 0–360, where 0 corresponds to red and 60 corresponds to yellow.

SPECTROPHOTOMETRY

To evaluate differences in light environments between the two populations, we measured light transmission spectra in the 350–750 nm range, using an AvaSpec 2048 spectrophotometer with a 10-m fibre cable (100 µm) and SPECTRAWIN, version 4.16 (Avantes). Measurements were taken in the shade, between 08.50 h and 09.00 h in the morning. At Makobe (1 December 2002), we measured light intensity in 1-m steps down to a depth of 10 m. At Kissenda (26 November 2002), we measured light intensity in 50-cm steps down to a depth of 3 m. The light intensity below a depth of 3 m was too low for spectrophotometry.

STATISTICAL ANALYSIS

All analyses were carried out in R software (R-Development-Core-Team, 2008). Results of the

mate choice experiments were analysed in generalized linear models. For every male courtship display event, we recorded whether the female responded by approaching. Female approach frequencies were compared between the two males in a pair using binomial models with logit link functions (using the raw frequency data rather than calculating proportions). We tested whether females had significant preferences for those males in the pairs that were largest (standard length) or that had the highest colour scores (red, yellow, black). For each of these male characters, we also tested whether female preference increased with increasing differences between the males in a pair. Effects of male traits on female preference were tested for significance using *F*-tests; test statistics were adjusted for over- and underdispersion (Venables & Ripley, 2002). To compare the two populations, we added 'population' as a factor to the models. We separately report the results for each courtship display: Lateral Display, Quiver and Lead Swim. For the population comparison, we use female response to male Lateral Display, which is the most frequent display (results for other displays were qualitatively similar).

We compared male traits between islands using *t*-tests for data that were normally distributed (male size) and Mann–Whitney *U*-tests for data that were not normally distributed data (colour scores).

RESULTS

FEMALE PREFERENCES

Makobe females (from clear water; Maan *et al.*, 2004) showed highly significant preferences for males with high redscores (effect of male redscore difference on female differential response to male Lateral Display, Quiver and Lead Swim: all $F > 10.93$, $P < 0.002$). Females also preferred males with high yellowscores (all $F > 4.72$, $P < 0.035$) and a weak trend indicated preferences for high blackscore (Lateral Display only, $F_{1,46} = 2.91$, $P = 0.095$). Females tended to prefer smaller males ($3.48 < F < 5.25$, $0.027 < P < 0.069$). After accounting for the effect of male redscore, however, none of these other male traits significantly affected preference. Finally, females showed stronger preferences for larger redscore differences between males (Lateral Display: $F_{1,47} = 20.56$, $P = 0.004$; Quiver: $F_{1,43} = 13.12$, $P = 0.011$, not significant for Lead Swim).

For Kissenda females (from turbid water), we found that the response to male display behaviour did not differ between trials with Kissenda males and trials with Makobe males (all $F < 0.042$, $P > 0.84$) and we therefore included all trials in the analyses. Females responded significantly more to the Lateral Display

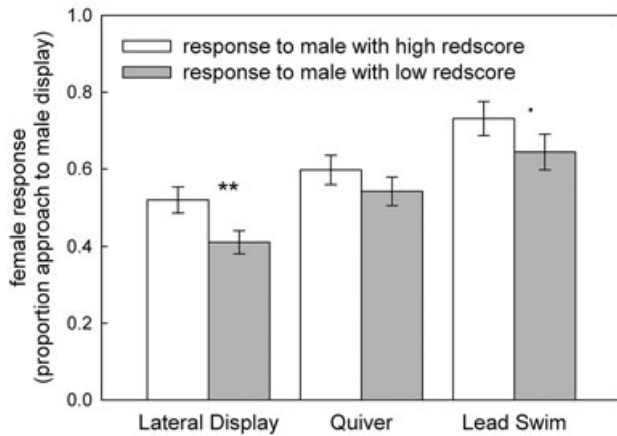


Figure 2. Preferences of Kissenda females (from turbid water) for male redscore. Bars indicate the average female response (proportion of male display followed by female approach) toward the two males in a pair, with standard errors. Females responded more frequently to the Lateral Display of the male with the higher redscore. A similar trend was observed for Lead Swim.

of the male in a pair with the higher redscore ($F_{1,41} = 10.86$, $P = 0.0021$; Fig. 2) and there was a trend in the same direction for response to Lead Swim ($F_{1,34} = 3.86$, $P = 0.058$; there was no significant preference in response to male Quiver: $F_{1,41} = 1.19$, $P = 0.28$). By contrast to Makobe females, Kissenda females did not show stronger preferences for larger differences in redscore between the two males in a pair; a weak trend indicated that preferences for Lateral Display actually decreased with increasing redscore difference ($F_{1,40} = 3.35$, $P = 0.074$; responses to Quiver and Lead Swim: $F < 1.86$, $P > 0.18$). Redscore was the only significant determinant of female preferences, there were no effects of other male traits (yellowscore, blackscore, body size: all $F < 2.38$, $P > 0.13$).

COMPARING FEMALE PREFERENCES BETWEEN ISLANDS

Makobe and Kissenda females responded similarly to the experimental paradigm: the proportion of male displays that was followed by female approach did not differ between populations ($F < 0.33$, $P > 0.57$ for all displays). As shown in Figure 3, females from Kissenda (turbid water) showed significantly weaker preferences for all measured male colour traits than females from Makobe (clear water). Preference for the reddest male in a pair was overall highly significant ($F > 8.77$, $P < 0.0041$ for all displays), although this preference was weaker among Kissenda females (Lateral Display: $F_{1,88} = 4.69$, $P = 0.033$). Similarly, despite an overall preference for males with high

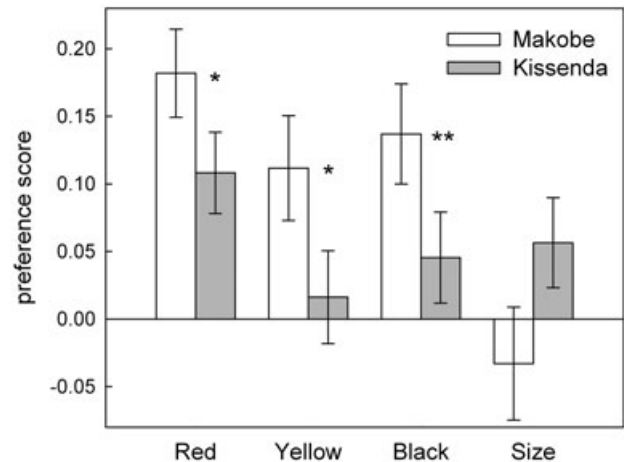


Figure 3. Comparison of preference behaviour between females from Makobe (clear water, open bars; data from Maan *et al.*, 2004) and Kissenda (turbid water, grey bars). Preferences are expressed as the difference in female response (proportion of approach to male Lateral Display) between the two males in a pair. Bars indicate preference means with standard errors. Positive values indicate preferences for males with high colour scores (red, yellow, and black) or large size (i.e. standard length). Makobe females showed significantly stronger preferences for all three measured components of male coloration: red, yellow, and black. The difference in preference for large male size was not significant.

yellowscores ($2.77 < F < 5.99$, $0.016 < P < 0.099$ for all displays), preferences of Kissenda females were significantly weaker ($F_{1,88} = 3.97$, $P = 0.049$). Finally, the overall preference for males with high blackscores was significant ($F_{1,89} = 4.27$, $P = 0.042$), although significantly weaker in Kissenda females ($F_{1,88} = 7.62$, $P = 0.0070$). Females did not prefer larger males ($F_{1,89} = 0.12$, $P = 0.73$) and this did not differ between populations ($F_{1,88} = 2.46$, $P = 0.12$).

COMPARING MALE COLORATION BETWEEN ISLANDS

Wildcaught males from Makobe (clear water) had higher redscores than those from Kissenda (turbid water): $N_1 = 28$, $N_2 = 17$ (Mann–Whitney U -test: $Z = 3.30$, $P = 0.001$; Fig. 4). Moreover, dorsal hue was significantly different between populations: Kissenda males had higher hue values (i.e. were more orange) than Makobe males (Mann–Whitney U -test: $Z = 2.46$, $P = 0.014$). These two measures are independent: the range of dorsal hues in both populations was well within the chosen redscore criteria. Yellowscore did not differ between populations ($Z = -0.40$, $P = 0.69$). Blackscore tended to be higher among Makobe males ($Z = 1.71$, $P = 0.087$). Kissenda males were significantly larger than Makobe males [standard length:

Kissenda ($N = 17$): 90.6 ± 1.6 mm; Makobe ($N = 28$): 80.9 ± 0.5 mm; $t = -5.64$, $P < 0.001$; weight: Kissenda: 19.1 ± 0.7 g; Makobe: 17.7 ± 0.4 g; $t = -5.56$, $P < 0.001$. By selecting the largest Kissenda males (see Material and methods), we could have introduced a bias, although it is shown in Figure 4E that even

the largest males of Makobe were smaller than the average size in the Kissenda sample.

LIGHT TRANSMISSION AT MAKOBE AND KISSEDA ISLANDS

The decline of light intensity with increasing depth was steeper in the turbid waters of Kissenda than in the clearer waters of Makobe (Fig. 5): the overall light intensity in the Kissenda *P. nyererei* habitat (2–5 m depth) was less than 67% of that at Makobe (4–7 m). Given that spectrophotometry was not possible beyond 3 m depth at Kissenda, the actual differences between the two *P. nyererei* habitats are most likely even greater.

DISCUSSION

Previous work has demonstrated that, in relatively clear water, male coloration in *P. nyererei* is subject to directional sexual selection by female choice (Maan *et al.*, 2004). In the present study, we reproduce this finding for a second population: also at Kissenda, of the four measured traits, red coloration is the most important determinant of female mate preferences. Given that male coloration not only affects intraspecific sexual selection, but also mediates assortative mating between *P. nyererei* and its close relative *P. pundamilia* (with blue nuptial coloration; Seehausen & Van Alphen, 1998), this result is consistent with the hypothesis that directional sexual selection on male coloration has contributed to the divergence of the two species.

Despite a shared preference for male red coloration, however, the two populations of *P. nyererei* showed marked differences in preference behaviour. Females from the clear waters of Makobe Island had stronger preferences for all aspects of male coloration: red, yellow, and black. Preferences for male size did not differ significantly between populations, although a larger sample size, or larger size differences between

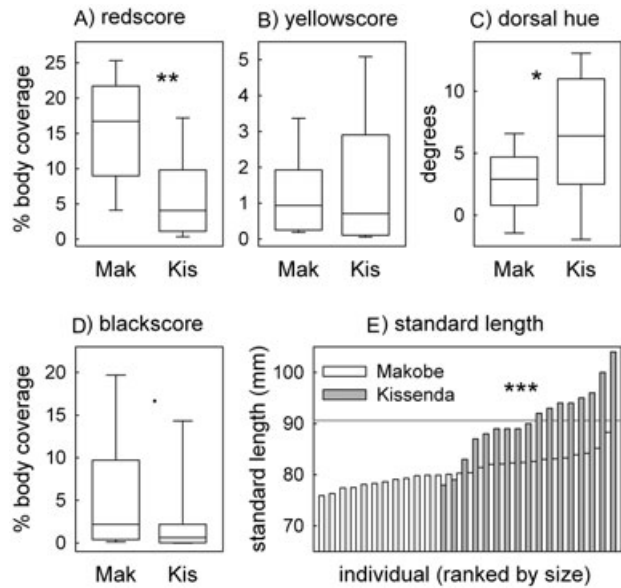


Figure 4. Nuptial coloration and body size of wild-caught males from Makobe (Mak, $N = 28$; clear water) and Kissenda (Kis, $N = 17$; turbid water). Boxes represent 75% confidence intervals intercepted by the median, error bars indicate 90% confidence intervals. A, Makobe males had significantly higher redscores than Kissenda males. B, yellowscore did not differ between populations. C, Makobe and Kissenda males differed significantly in dorsal hue: Kissenda males were more orange, whereas Makobe males were more red. D, Makobe males tended to have higher blackscores than Kissenda males. E, Kissenda males were significantly larger than Makobe males. The grey line indicates the average standard length of Kissenda males.

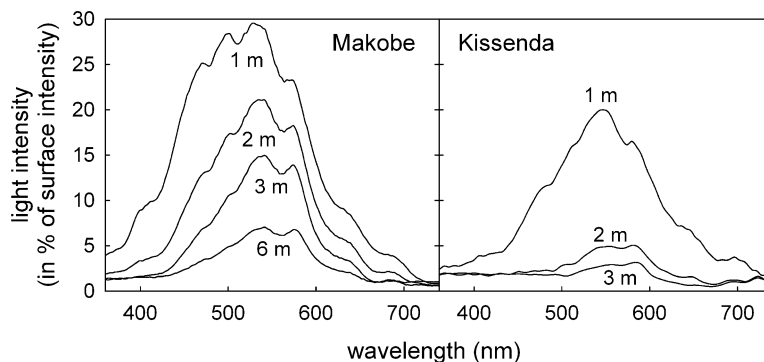


Figure 5. Underwater light spectra at different depths at the islands Makobe (clear water) and Kissenda (turbid water).

stimulus males, might have revealed a preference for larger males among Kissenda females (Fig. 3).

We tested both populations in identical laboratory settings, with clear water and broad-spectrum illumination. The finding of significant preference differences under these conditions indicates that the observed relation between male coloration and water transparency (Seehausen *et al.*, 1997; Maan, 2006) is not mediated by environmental variation in signal transduction alone. Rather, it suggests that female mating preferences have changed in response to this variation. Although investigation of additional populations is warranted, the present study provides the first evidence for intraspecific preference-trait co-evolution in cichlid fish, confirming that the decrease in Lake Victoria water transparency affects both interspecific mate choice and intraspecific sexual selection (Seehausen *et al.*, 1997).

The differences in preference correspond reasonably well to the differences in male traits: Kissenda males had significantly lower redscores, tended to have lower blackscores, and were significantly larger than Makobe males. Together with a more orange dorsal hue, Kissenda males have an overall lighter appearance (Fig. 1B).

Several mechanisms may explain how environmental variation promotes divergence in sexual communication. In the relatively dark light environment of Kissenda Island, selection for conspicuous males may favour lighter colour and larger body size. Although the spectral properties of Lake Victoria waters promote long-wavelength rather than short-wavelength signals towards deeper water (Maan *et al.*, 2006a), low light transmission in turbid locations will constrain the range of wavelengths that produce a signal of sufficient intensity. The water transparency at Kissenda may approach threshold conditions in terms of permitting long-wavelength colour signals, even at moderate water depths. Indeed, red phenotypes are rare in *Pundamilia* populations that inhabit even murkier waters (Seehausen *et al.*, 2008).

Environmental effects on male trait expression may be followed by changes in female preferences as a result of Fisherian coevolution. Alternatively, trait divergence is initiated by changes in female mate choice. Environmental conditions affect the cost and benefits of female choosiness (Candolin & Heuschele, 2008). The light conditions in turbid water may hamper mate detection, reduce overall choosiness, and demote coloration as a mate choice cue. Moreover, previous work indicated that female preferences for red males may select for high-quality mates: in the Makobe population, males with high redscores had lower parasite loads (Maan *et al.*, 2006b). The red and yellow colours of *P. nyererei* are produced by caro-

tenoids, with higher concentrations yielding more reddish hues (Maan *et al.*, 2006b). The lower redscores and more orange hues of Kissenda males probably correspond to lower carotenoid content, possibly constituting a less costly display. We lack information about carotenoid availability in the two populations, although the difference in coloration persists in laboratory-bred fish (Seehausen *et al.*, 1997; Ole Seehausen (OS) and Martine Maan (MEM)), indicating a genetic component. Therefore, selection for lighter colours exerted by the darker visual environment could lead to reduced reliability of the colour signal, making weaker colour preferences adaptive (Grether, 2000). Further experiments are necessary to tease apart the effects on female preference of the different components of male coloration, such as hue, brightness, and contrast, as well as their value as indicators of quality.

When colour assessment is hampered by low water transparency, or unreliable as a result of low production costs, females may use alternative cues. Recent studies indicate that olfactory cues may be important in cichlid mate choice (Plenderleith *et al.*, 2005), possibly because they provide information about major histocompatibility complex compatibility (Blais *et al.*, 2007). Similarly, male body size may signal quality (Dijkstra, Seehausen & Groothuis, 2005) and could replace coloration as a mate choice criterion. By contrast to the red coloration of *P. nyererei*, however, body size is not a species-specific trait. In fact, males of the closely-related species *P. pundamilia* are generally larger than *P. nyererei* males (Seehausen, 1996). As a result, preferences of *P. nyererei* females for larger males could lead to interbreeding with *P. pundamilia*.

Because we used wildcaught fish in these experiments, preferences could have been influenced by the environment in which the fish developed. For example, fish colour vision can be phenotypically plastic (Fuller *et al.*, 2005; Shand *et al.*, 2008), possibly influencing colour preferences in a mate choice context. In addition, female preferences may be influenced by the male phenotypes they encounter in nature (Verzijden & ten Cate, 2007). Although these nongenetic mechanisms could affect the strength and direction of selection on male traits, they may not persist when environmental conditions change.

Our experimental design focused on testing within-population preferences, which precludes critical evaluation of the alternative hypothesis that preference differences are a result of differences in male traits, rather than vice versa. Although Kissenda females did not respond differently to native and Makobe males, we should be careful to conclude that the current pattern of male trait variation is the direct result of differences in sexual selection. The

results obtained, however, do show that, presently, male traits are subject to different sexual selection pressures in the two study populations.

It is unlikely that weaker female preferences are the result rather than cause of introgression of *P. pundamilia* genes into *P. nyererei*. If introgression is responsible for the significant intraspecific preference differences documented in the present study, we would expect it to also affect preferences (and male colours) at the interspecific level. This does not appear to be the case: the distribution of coloration among *Pundamilia* males is strongly bimodal at Kissenda (Magalhaes *et al.*, 2009; Seehausen *et al.*, 2008), and Kissenda *P. nyererei* females show significant interspecific preferences for red males (Dijkstra, Van der Zee & Groothuis, 2008). Strong bimodality in colour and preference is also observed at the very similar *P. nyererei* population at Python Island (Seehausen & Van Alphen, 1998; Seehausen *et al.*, 2008). These patterns suggest that the differences in intraspecific female mating preferences between Kissenda and Makobe are not a result of admixture.

The present study contributes to a growing number of studies indicating that variation in water transparency affects sexual selection in fish (Luyten & Liley, 1991; Järvenpää & Lindström, 2004; Heubel & Schlupp, 2006; Engstrom-Ost & Candolin, 2007; Wong, Candolin & Lindstrom, 2007). In Lake Victoria cichlids, colour signals mediate both intraspecific sexual selection and interspecific reproductive isolation. As a result, changes in visual communication between males and females of one species may have severe consequences at the interspecific level (Seehausen *et al.*, 1997; Taylor *et al.*, 2006). Therefore, the results obtained in the present study emphasize the importance of measures for counteracting the ongoing eutrophication of Lake Victoria.

ACKNOWLEDGEMENTS

We thank the Tanzanian Commission for Science and Technology for research permission and the Tanzanian Fisheries Research Institute (Professor Philip Bwathondi and Egid Katunzi) for hospitality and facilities. Mhoja Kayeba and Mohammed Haluna provided technical assistance in the field. We thank John Mrosso, Peter Hiddinga, Roelof van der Kleij, and Kees Hofker for their help with spectrophotometry and Martin Brittijn for the map of the Mwanza Gulf. Tom Van Dooren, Katja Heubel, and two anonymous reviewers provided helpful comments on the manuscript. The study was supported by the Netherlands Science Foundation (WOTRO 82-243 to O.S. and J.J.M.V.A.) and the Dobberke Foundation (to M.E.M.).

REFERENCES

- Badyaev AV, Hill GE, Weckworth BV. 2002.** Species divergence in sexually selected traits: increase in song elaboration is related to decrease in plumage ornamentation in finches. *Evolution* **56**: 412–419.
- Blais J, Rico C, van Oosterhout C, Cable J, Turner GF, Bernatchez L. 2007.** MHC adaptive divergence between closely related and sympatric African cichlids. *PLoS ONE* **2**: e734.
- Boughman JW. 2001.** Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature* **411**: 944–948.
- Candolin U, Heuschele J. 2008.** Is sexual selection beneficial during adaptation to environmental change? *Trends in Ecology & Evolution* **23**: 446–452.
- Candolin U, Salesto T, Evers M. 2007.** Changed environmental conditions weaken sexual selection in sticklebacks. *Journal of Evolutionary Biology* **20**: 233–239.
- Dijkstra PD, Seehausen O, Groothuis TGG. 2005.** Direct male–male competition can facilitate invasion of new colour types in Lake Victoria cichlids. *Behavioral Ecology and Sociobiology* **58**: 136–143.
- Dijkstra PD, Van der Zee EM, Groothuis TGG. 2008.** Territory quality affects female preference in a Lake Victoria cichlid fish. *Behavioral Ecology and Sociobiology* **62**: 747–755.
- Endler JA. 1990.** On the measurement and classification of color in studies of animal color patterns. *Biological Journal of the Linnean Society* **41**: 315–352.
- Endler JA, Houde AE. 1995.** Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution* **49**: 456–468.
- Engstrom-Ost J, Candolin U. 2007.** Human-induced water turbidity alters selection on sexual displays in sticklebacks. *Behavioral Ecology* **18**: 393–398.
- Fisher HS, Wong BBM, Rosenthal GG. 2006.** Alteration of the chemical environment disrupts communication in a freshwater fish. *Proceedings of the Royal Society of London Series B, Biological Sciences* **273**: 1187–1193.
- Fuller RC. 2002.** Lighting environment predicts the relative abundance of male colour morphs in bluefin killifish (*Lucania goodei*) populations. *Proceedings of the Royal Society of London Series B, Biological Sciences* **269**: 1457–1465.
- Fuller RC, Carleton KL, Fadool JM, Spady TC, Travis J. 2005.** Genetic and environmental variation in the visual properties of bluefin killifish, *Lucania goodei*. *Journal of Evolutionary Biology* **18**: 516–523.
- Grether GF. 2000.** Carotenoid limitation and mate preference evolution: a test of the indicator hypothesis in guppies (*Poecilia reticulata*). *Evolution* **54**: 1712–1724.
- Heubel KU, Schlupp I. 2006.** Turbidity affects association behaviour in male *Poecilia latipinna*. *Journal of Fish Biology* **68**: 555–568.
- Järvenpää M, Lindström K. 2004.** Water turbidity by algal blooms causes mating system breakdown in a shallow-water fish, the sand goby *Pomatoschistus minutus*. *Proceedings of the Royal Society of London Series B, Biological Sciences* **271**: 2361–2365.

- Knight ME, Turner GF. 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 of London Series B, Biological Sciences* **271**: 675–680.
- Luyten PH, Liley NR. 1991.** Sexual selection and competitive mating success of male guppies (*Poecilia reticulata*) from 4 Trinidad populations. *Behavioral Ecology and Sociobiology* **28**: 329–336.
- Lythgoe JN. 1984.** Visual pigments and environmental light. *Vision Research* **24**: 1539–1550.
- Maan ME. 2006.** *Sexual selection and speciation: mechanisms in Lake Victoria cichlid fish*. PhD Thesis, University of Leiden.
- Maan ME, Hofker KD, van Alphen JJM, Seehausen O. 2006a.** Sensory drive in cichlid speciation. *American Naturalist* **167**: 947–954.
- Maan ME, Seehausen O, Söderberg L, Johnson L, Ripmeester EAP, Mrosso HDJ, Taylor MI, van Dooren TJM, van Alphen JJM. 2004.** Intraspecific sexual selection on a speciation trait, male coloration, in the Lake Victoria cichlid *Pundamilia nyererei*. *Proceedings of the Royal Society of London Series B, Biological Sciences* **271**: 2445–2452.
- Maan ME, Van der Spoel M, Quesada Jimenez P, Van Alphen JJM, Seehausen O. 2006b.** Fitness correlates of male coloration in a Lake Victoria cichlid fish. *Behavioral Ecology* **17**: 691–699.
- Magalhaes IS, Mwaiko S, Schneider MV, Seehausen O. 2009.** Divergent selection and phenotypic plasticity during incipient speciation in Lake Victoria cichlid fish. *Journal of Evolutionary Biology* **22**: 260–274.
- Marshall NJ. 2000.** Communication and camouflage with the same 'bright' colours in reef fishes. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **355**: 1243–1248.
- Pauers MJ, McKinnon JS, Ehlinger TJ. 2004.** Directional sexual selection on chroma and within-pattern colour contrast in *Labeotropheus fuelleborni*. *Proceedings of the Royal Society of London Series B, Biological Sciences* **271**: S444–S447.
- Plenderleith M, van Oosterhout C, Robinson RL, Turner GF. 2005.** Female preference for conspecific males based on olfactory cues in a Lake Malawi cichlid fish. *Biology Letters* **1**: 411–414.
- R-Development-Core-Team. 2008.** *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.
- Seehausen O. 1996.** *Lake Victoria Rock Cichlids; taxonomy, ecology and distribution*. Verduijn: Zevenhuizen.
- Seehausen O, Terai Y, Magalhaes IS, Carleton KL, Mrosso HDJ, Miyagi R, Van der Sluijs I, Schneider MV, Maan ME, Tachida H, Shichida Y, Imai H, Okada N. 2008.** Speciation through sensory drive in cichlid fish. *Nature* **455**: 620–626.
- Seehausen O, Van Alphen JJM. 1998.** The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei* complex). *Behavioral Ecology and Sociobiology* **42**: 1–8.
- Seehausen O, Van Alphen JJM, Witte F. 1997.** Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science* **277**: 1808–1811.
- Shand J, Davies WL, Thomas N, Balmer L, Cowing JA, Pointer M, Carvalho LS, Trezise AEO, Collin SP, Beazley LD, Hunt DM. 2008.** The influence of ontogeny and light environment on the expression of visual pigment opsins in the retina of the black bream, *Acanthopagrus butcheri*. *Journal of Experimental Biology* **211**: 1495–1503.
- Slabbekoorn H, Peet M. 2003.** Birds sing at a higher pitch in urban noise – great tits hit the high notes to ensure that their mating calls are heard above the city's din. *Nature* **424**: 267–267.
- Taylor EB, Boughman JW, Groenenboom M, Sniatynski M, Schluter D, Gow JL. 2006.** Speciation in reverse: morphological and genetic evidence of the collapse of a three-spined stickleback (*Gasterosteus aculeatus*) species pair. *Molecular Ecology* **15**: 343–355.
- Taylor PW, Jackson RR. 1999.** Habitat-adapted communication in *Trite planiceps*, a New Zealand jumping spider (Araneae, Salticidae). *New Zealand Journal of Zoology* **26**: 127–154.
- Venables WN, Ripley BD. 2002.** *Modern applied statistics with S*. New York, NY: Springer-Verlag.
- Verschuren D, Johnson TC, Kling HJ, Edgington DN, Leavitt PR, Brown ET, Talbot MR, Hecky RE. 2002.** History and timing of human impact on Lake Victoria, East Africa. *Proceedings of the Royal Society of London Series B, Biological Sciences* **269**: 289–294.
- Verzijden MN, ten Cate C. 2007.** Early learning influences species assortative mating preferences in Lake Victoria cichlid fish. *Biology Letters* **3**: 134–136.
- Wong BBM, Candolin U, Lindstrom K. 2007.** Environmental deterioration compromises socially enforced signals of male quality in three-spined sticklebacks. *American Naturalist* **170**: 184–189.