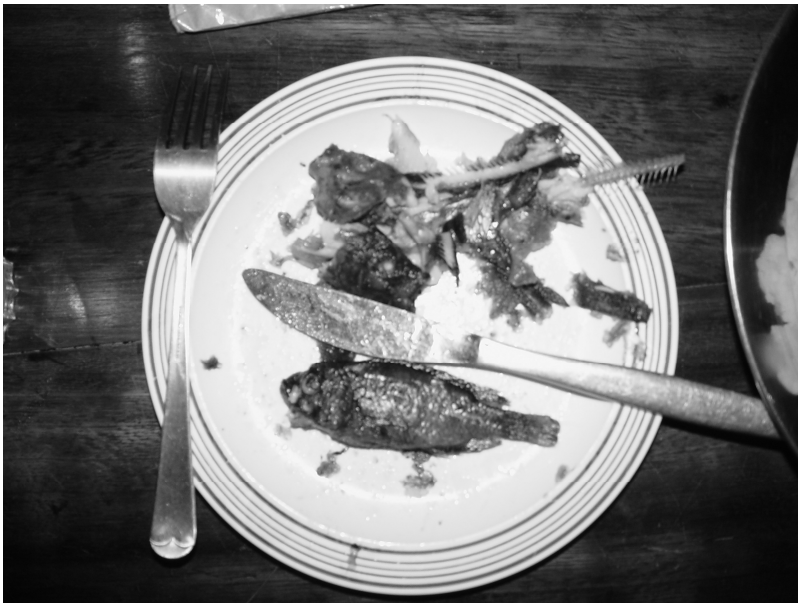
Sexual selection and speciation: mechanisms in Lake Victoria cichlid fish

Maan, M.E.

Chapter 6

'The more diversified the descendants from any one species become in structure, constitution, and habits, by so much will they be better enabled to seize on many and widely diversified places in the polity of nature, and so be able to increase in numbers.'

Darwin, 1859



Female mating preferences and male coloration co-evolve in response to water transparency in a Lake Victoria cichlid fish

Martine E. Maan, Ole Seehausen and Jacques J.M. van Alphen

(submitted)

The Lake Victoria cichlid species flock is a classic example of rapid speciation that may be driven by sexual selection: female choice for male nuptial coloration exerts sexual selection within species and maintains reproductive isolation between species. Recently however, water transparency in Lake Victoria has been declining, with critical consequences for cichlid diversity: low water transparencies coincide with increasing rates of hybridisation between closely related species, decreasing species numbers and less colourful fish. Here, we investigate a possible mechanism underlying this pattern: increased turbidity may lead to relaxation of sexual selection on male nuptial coloration within species, affecting both male nuptial coloration and the rate of interspecific hybridisation. We study *Pundamilia nyererei*, a species known to interbreed with a sister species in turbid water, but not in clear water. We compare measures of intraspecific sexual selection between two populations from locations that differ in water transparency. In clear water, female *P. nyererei* exert directional sexual selection on male red coloration. Here we find that in the population from turbid water, female preference for male red coloration is significantly weaker. Further, we find that both the amount and the hue of male red coloration differ significantly between populations, consistent with adaptation to the different photic habitats of the two populations. These findings suggest that the observed correlation between male coloration and water transparency is not mediated by environmental variation alone. Rather, they indicate that female mating preferences have evolved in response to this variation. This is the first evidence for intraspecific preference-trait co-evolution in cichlid fish. Our findings underline the importance of measures to counteract the ongoing eutrophication of Lake Victoria.

Introduction

Animal communication strongly depends on the signal propagation properties of the environment. Animals have been shown to adjust their sexual signals accordingly, trading off different components of sexual display in response to environmental variation. Differential employment or elaboration of visual, acoustic, seismic or behavioural displays has been demonstrated both at the intraspecific (e.g. spiders: Taylor & Jackson 1999) and interspecific level (e.g. birds: Badyaev et al. 2002). Also human-induced changes in the environment influence the nature and effectiveness of animal signals (Seehausen et al. 1997a; Slabbekoorn & Peet 2003).

In water, visual communication is constrained by the light transmission properties of the water and its organic and inorganic content: water turbidity can severely hamper sexual selection on visual cues (Järvenpää & Lindström 2004). Besides overall turbidity, the wavelength distribution of the transmitted light affects the visibility of colour signals. First, the ambient light spectrum determines the colour of the background against which a signal is observed, influencing its conspicuousness (e.g. Marshall 2000; Fuller 2002). Second, the spectrum determines the 'colour space' available for reflection and perception (Seehausen et al. 1997a). 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).

In Lake Victoria cichlid fish, female mate choice for male nuptial coloration plays an important role in the evolution and maintenance of an exceptional species diversity (Dominey 1984; Seehausen 2000). Due to human population growth that entails increased deforestation and nutrient runoff, water transparency in the lake has been declining for several decades (Verschuren et al. 2002). Because many haplochromine cichlids rely on visual cues for mate recognition (Seehausen & Van Alphen 1998; Knight & Turner 2004), increased turbidity poses a threat to species diversity (Seehausen et al. 1997a).

Pundamilia pundamilia and *Pundamilia nyererei* are two closely related species that occur sympatrically at several islands in south-eastern Lake Victoria. They are morphologically similar, but differ in male nuptial coloration: male *P. pundamilia* are metallic blue and male *P. nyererei* are bright red and yellow. Females of both species are cryptically coloured. Reproductive isolation is maintained by female choice for different male nuptial coloration (Seehausen & Van Alphen 1998), but the two phenotypes hybridise in locations with low water transparency. In extremely turbid water (Secchi disk reading below 50 cm), populations consist of hybrids only (Seehausen et al. 1997a). Further, the brightness of *P. nyererei* male nuptial coloration decreases as the water becomes more turbid (Seehausen et al. 1997a).

The mechanism by which transparency affects male coloration is unknown: i) female mating preferences may evolve in response to variation in water transparency, or ii) the strength of selection on male coloration by female mating preferences may be a direct function of signal transduction through the water, without

mating preferences evolving themselves. Here, we investigate these alternatives. Previous work has shown that in a clear water population of *P. nyererei*, female choice exerts directional sexual selection on male red coloration (Chapter 2). In the present study, we investigate the mate choice behaviour of a *P. nyererei* population from turbid water, using the same laboratory set-up as before to allow for comparison of the two populations. To test whether female choice, male coloration and ambient light spectrum covary, we measure the light spectrum in both habitats and we quantify the variation in male coloration in both populations.

Methods

Fish

Male *Pundamilia nyererei* are bright red dorsally and have yellow sides intercepted by black vertical bars. Females are cryptically brown with dark vertical bars. We compare *P. nyererei* populations from two islands in south-eastern Lake Victoria that differ in water transparency: Kissenda (Secchi disk reading mean±se =67.5±1.4 cm, n=4, in the study period) and Makobe (221±15 cm; n=29, Figure 6.1a). Hybrids between *P. nyererei* and *P. pundamilia* have never been observed at Makobe Island, but are regularly encountered at Kissenda (in 2001-2003, we caught 1 hybrid male with every 15 *P. nyererei* males, n=79 fish, unpublished data; see also Taylor et al. subm.). At Makobe, *P. nyererei* territories are most abundant between four and seven meters depth and fish were collected by gillnetting. At Kissenda, *P. nyererei* predominantly occurs between two and five meters depth and fish were collected by gillnetting and hook and line. In both populations, fish were collected between December 2000 and April 2001 and mate-choice experiments were carried out between November 2001 and August 2002. In the lake, *P. nyererei* males defend territories on the rocky bottom and attract females by vigorous courtship displays that resemble those of other haplochromine species (See-

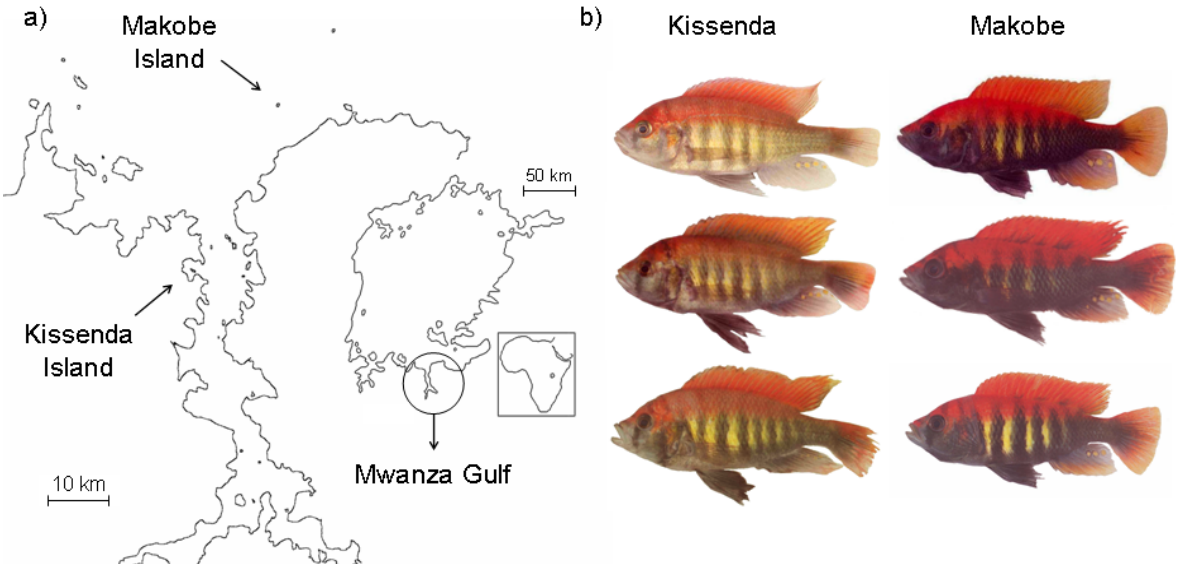


Figure 6.1 a) Lake Victoria and the locations of Kissenda and Makobe Islands.
b) *Pundamilia nyererei* males from both islands.

hausen & 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). Mating occurs in rocky crevices. After spawning, females mouthbrood eggs and larvae for about three weeks and guard the fry for an additional week after releasing them (Seehausen 1996).

Spectrophotometry

Light transmission spectra were determined in the 350-750 nm range, using an AvaSpec 2048 spectrophotometer with a 10-meter fibre cable (100 micron) and SpectraWin 4.16 software (Avantes). Measurements were taken in the shadow, between 8h50 and 9h00 in the morning, on November 26th 2002 at Kissenda Island and on December 1st 2002 at Makobe.

Mate choice experiment in the laboratory

Two-way mate choice experiments were carried out following the methods of Chapter 2: each male was kept in one of two six-sided perspex enclosures (140 litres) on either side of an 1800-litres aquarium. In each enclosure, shelter was provided by a brick on top of a PVC tube. These shelters were readily accepted by the males as the centres of their territories. In the middle of the main tank two large stones provided shelter for the test female and ensured that the males could not see each other. The water in the male enclosures was filtered internally, making chemical communication impossible. Air stones were present in both enclosures and in the main tank; water temperature was maintained at 24-26 °C.

We used 10 wildcaught *P. nyererei* males from Kissenda Island, assembled into 5 male pairs of fixed composition, and 9 Kissenda females. All males were photographed, measured and weighed. Redscore differences (see below) between Kissenda males in a pair ranged from 3 to 12% body coverage. Four Makobe males were used to assemble two additional pairs with large redscore difference: 9% and 24.8% body coverage. Size differences between paired males were below 7% of the average size of the two males in a pair.

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 at least two hours separated consecutive trials. Male pairs were exchanged after three successful trials or after two days. The position of the males (left or right side of the aquarium) was reversed each time the same pair was introduced. To start a trial, the test female was released in the middle of the tank. Behaviour was recorded with Observer 3.0 software (Noldus). 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 an interaction threshold distance. Trials were completed once 15 minutes of observation time had been collected.

Each female was tested once with every male pair, but in the analysis we included only those trials in which both males courted (performing Quiver or Lead Swim at least once) and the test female responded positively (approaching a male in response to his Lateral Display, Quiver or Lead Swim) to at least one of them (32 trials with Kissenda males; 11 trails with Makobe males). For every male courtship display event, we recorded whether the female responded by approaching the male. The resulting proportion is our measure of female response to each male. The difference in female response to two males in a trial is our measure of female preference. We also recorded aggressive behaviour of the males (butting and biting attempts) and counted the total number of encounters of each male with the test female. The results of this experiment are compared to a previous mate-choice experiment carried out with Makobe males and females (Chapter 2).

Male coloration at Makobe and Kissenda Islands

To quantify male colour variation in the field, we collected mature males from both islands. At Makobe Island, we collected 28 males that were territorial and sexually active, as established during observations using SCUBA. Water transparency at Kissenda Island does not allow for underwater observations. Therefore, we collected 17 males that expressed red, yellow and black nuptial coloration and that belonged to the largest 10% of the population. Immediately after capture, males were photographed for colour analysis and sacrificed on melting ice. They were subsequently measured (standard length, SL, to the nearest 0.1 mm) and weighed (W, to the nearest 0.1 g). We calculated condition factor (CF) as $CF=100\cdot(W/SL^3)$ (Sutton et al. 2000).

Colour analysis

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. We used an SLR camera and two flashes on either side and subsequently digitised 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 4.0 (SPSS Inc.). We defined criteria delimiting the body area (excluding fins and eyes) covered by red and yellow by a combination of hue (RGB) and saturation (red: hue = 0-26 plus 232-255, saturation 40-97%; yellow: hue = 27-45, saturation 40-97%) and subsequently calculated the area of the fish body that matched these criteria, yielding a percentage of body coverage, subsequently referred to as redscore and yellowscore. We similarly defined criteria for blackness to quantify the body coverage of the black vertical bars and ventral aspects of the body (intensity=0-75; blackscore). To compare male coloration between populations, we calculated an additional variable: dorsal hue. This is the average hue of the male dorsum, measured in five standardised positions, using the colour sampler tool in Adobe PhotoShop 6.0. Hue was defined as a location on the standard colour wheel, expressed as a degree between 0 and 360, where 0° corresponds to red and 60° corresponds to yellow.

Data analysis

Male and female behaviour in the mate choice experiments was analysed in generalised linear models (GLM) using R software (Ihaka & Gentleman 1996; http://www.r-project.org). We separately report the results for the three most important male courtship displays: Lateral Display, Quiver and Lead Swim. Female response to male courtship was analysed in Poisson models with log link functions. To compare female response between the two males in a pair, we used binomial models with logit link functions. We tested whether females had significant preferences for those males in the male 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 size or colour difference between the males in a pair. To control for random effects of individual females and male pairs, we included 'trial number' as a factor in all models. To compare the two populations, we added 'population' as a factor in the models. Significance levels were determined by F-tests examining the change in deviance following removal of a variable; test statistics were adjusted for over- and underdispersion (Venables & Ripley 2002).

Other statistical analyses were carried out in SPSS 10.0 (SPSS Inc.), using t-tests and Pearson correlations for normally distributed data, and Mann-Whitney-U tests and Spearman correlations for non-normally distributed data.

Results

Behaviour of Kissenda females

In the trials with Kissenda males and females, females showed a significant preference for the male in a pair with the highest redscore (Figure 6.2; Lateral Display estimate=0.819±0.149, $F_{1,31}$=30.93, p<0.0001, Quiver estimate=0.831±0.186, $F_{1,31}$=20.41, p<0.0001, Lead Swim estimate=1.057±0.311, $F_{1,26}$=12.23, p= 0.0017). Female preference did not increase with increasing redscore difference between the males (Lateral Display and Lead Swim: F<2.31, p> 0.14); differential

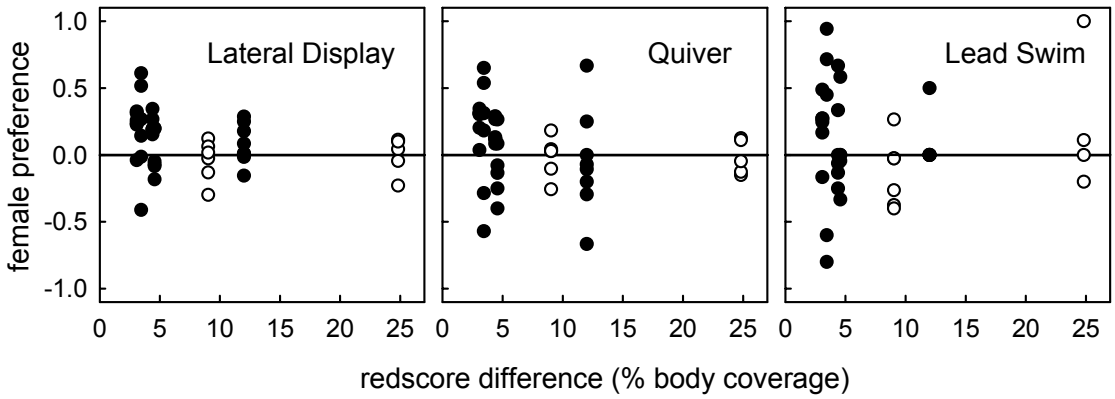


Figure 6.2 Female preference relative to the difference in redscore between the males in a pair, for each of the three courtship displays. Each symbol represents one trial; filled circles are trials with Kissenda males; open circles are trials with Makobe males.

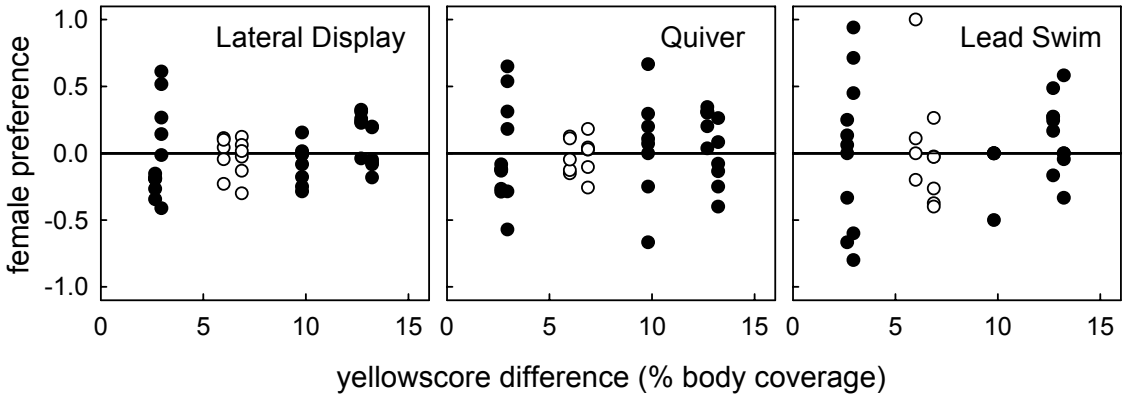


Figure 6.3 Female preference relative to the difference in yellowscore between the males in a pair, for each of the three courtship displays. Each symbol represents one trial; filled circles are trials with Kissenda males; open circles are trials with Makobe males.

response to male Quiver decreased with increasing redscore difference (estimate=-21.36±7.82, $F_{1,30}$=7.88, p=0.009).

The differences in colourscores and standard length between males in a pair were not correlated (Spearman correlations, r_s<0.6, p>0.10). Yet, females also significantly preferred the males with the highest yellowscores (Figure 6.3; Lateral Display estimate= 0.400±0.195, $F_{1,31}$=4.24, p=0.048, Quiver estimate= 0.736±0.200, $F_{1,31}$=13.92, p<0.001, Lead Swim estimate= 1.034±0.298, $F_{1,26}$=12.05, p=0.0018). For Lateral Display, there was a non-significant tendency for female preference to increase with increasing yellowscore difference between the males (estimate=7.500±4.039, $F_{1,30}$=3.44, p=0.073). For Quiver and Lead Swim, there were no such trends (F<1.38, p>0.25).

There were no significant preferences for male blackscore (F<1.42, p>0.24 for all displays). Finally, females responded more strongly to the Lateral Display of the larger male in a pair (estimate=0.470±0.190, $F_{1,31}$=6.15, p=0.019) but this was not significant for Quiver (estimate=0.442±0.222, $F_{1,31}$=3.99, p=0.055) and Lead Swim (estimate=0.185±0.349, $F_{1,26}$=0.28, p=0.60).

The minimal adequate GLM describing female response to male Lateral Display and Lead Swim included only the variable that described which of the males in a pair had the highest redscore. For female response to male Quiver, the magnitude of the difference in redscore remained in the model as well. The other variables, male yellowscore, blackscore and standard length did not significantly explain the remaining variation in female response.

The response of Kissenda females to male display behaviour did not differ between trials with Kissenda males and trials with Makobe males (all F<3.31, p>0.079). Yet, whereas the redscore differences in the two Makobe male pairs was larger than those in the Kissenda pairs, female preferences for male redscore were significantly weaker in trails with Makobe males (all F>5.84, p<0.022; Figure 6.2). Female preferences for male blackscore were slightly stronger in trials with Makobe males (4.10<F<4.30, 0.044<p<0.050). There were no differences in female response to male yellowscore or standard length (all F<3.93, p>0.054).

Comparison of Kissenda and Makobe experiments

In both series of intrapopulation trials, Makobe (Chapter 2) and Kissenda (this study), the total number of male displays and female positive responses were highly correlated ($F>41.6$, $p<<0.001$ for all displays, for both populations). Makobe males had higher display frequencies than Kissenda males ($F>5.22$, $p<0.025$ for all displays), but the relationship between male display and female response did not significantly differ between populations ($F<0.33$, $p>0.57$ for all displays). Preference consensus did not differ between populations either (Mann-Whitney-U test comparing the variance in female preference for 7 Makobe male pairs vs. 5 Kissenda male pairs: $Z<1.22$, $p>0.22$ for all displays). Likewise, the amplitude of female preference scores did not differ between populations ($F<3.36$, $p>0.071$ for all displays).

There was a significant difference between Kissenda and Makobe trials in the tendency of females to prefer the brighter red male in a pair (Figure 6.4). Pooling the data of both populations, there was a significant preference for redder males ($F>10.60$, $p<0.002$ for all displays), but this preference was weaker in Kissenda trials (significant for Lateral Display: estimate [interaction]=-7.88±3.73, $F_{1,77} = 4.62$, $p=0.035$ and for Lead Swim: estimate [interaction]=-20.68±8.21, $F_{1,68} =7.01$, $p=0.01$; not significant for Quiver: estimate [interaction]=-7.75±4.77, $F_{1,76} =2.71$, $p=0.10$). Conversely, there was an overall preference for males with high

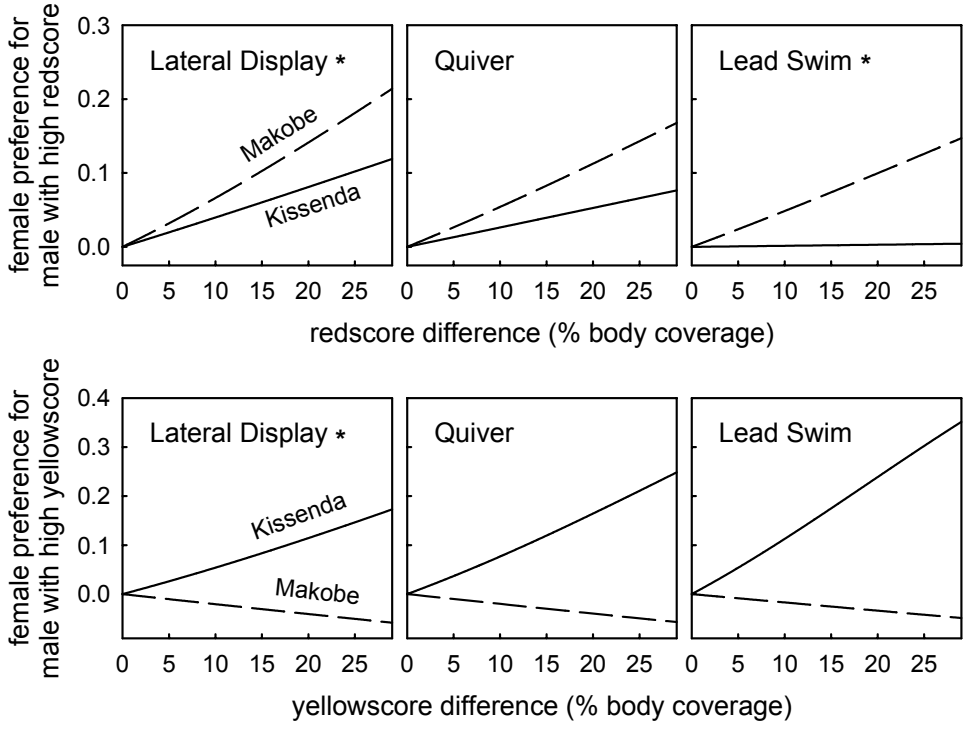


Figure 6.4 Preference curves (logit link functions) for Kissenda and Makobe females in the mate-choice experiments (intrapopulation trials only). Upper panel: female response to differences in male redscore; lower panel: female response to differences in male yellowscore. Curves are forced through the origin; asterisks denote significant differences in female response between populations.

yellowscores ($F>13.78$, $p<0.001$ for all displays), but this preference was stronger in Kissenda females (Lateral Display estimate [interaction]= 8.78±4.20, $F_{1,77}$= 4.44, p=0.038). The difference was not significant for Quiver and Lead Swim ($F<0.62$, $p>0.43$). There was no significant overall preference for high or low male blackscore ($F<2.11$, $p>0.15$), but whereas Makobe females tended to select against males with high blackscores, Kissenda females did not (interaction effects: $2.79<F<4.40$, $0.015<p<0.068$). Likewise, there was no overall preference for small or large males ($F<0.68$, $p>0.41$), but Kissenda females tended to prefer larger males whereas Makobe females did not (interaction effects: $2.05<F<4.28$, $0.017<p<0.14$).

Male coloration at Makobe and Kissenda Islands

Among wildcaught males, Makobe males had higher redscores than Kissenda males (n_1=28, n_2=17, MWU Z= -3.30, p=0.001; Figure 6.5). Yellowscore did not differ between populations (Z=-0.40, p=0.69; Figure 6.5). As a result, the yellow-to-red ratio was significantly higher in Kissenda males (median [range] Kissenda: 0.28 [0.01-5.49]; Makobe: 0.075 [0.01-0.73]; MWU Z=-2.44, p=0.015), giving the Kissenda males a more orange-ish appearance (Figure 6.1b). Moreover, the hue of male red coloration (dorsal hue) was significantly different between populations: Kissenda males had higher hue values, i.e. were more orange, than Makobe males (MWU Z=-2.46, p=0.014). These two measures are independent: the range of hues encountered in both populations was well within the chosen redscore criteria. 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±1.6mm; Makobe [n=28]: 80.9±0.5mm; t=-5.64, $p<0.001$; weight: Kissenda: 19.1±0.7g; Makobe: 17.7±0.4g; t=-5.56, $p<0.001$), but had a lower condition factor than Makobe males (Kissenda: 2.57±0.06; Makobe: 2.77±0.05; t=2.54, p=0.015). Condition factor was negatively related to

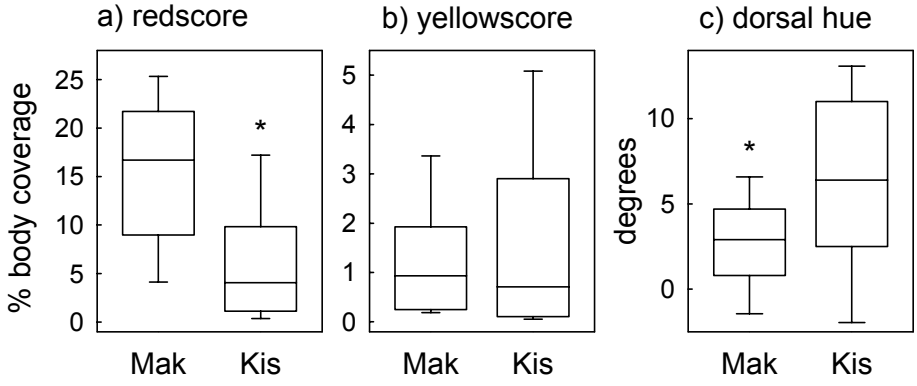


Figure 6.5 Nuptial coloration of wildcaught males from Makobe (Mak, n=28) and Kissenda (Kis, n=17). Boxes represent 75% confidence intervals intercepted by the median, error bars indicate 90% confidence intervals. a) Makobe males had significantly higher redscores than Kissenda males; b) yellowscore 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.

male redscore at Makobe (Pearson correlation, r=-0.44, p=0.02). At Kissenda Island this correlation was not significant (r=-0.37; p=0.14), but the relationship did not differ significantly between populations (GLM with condition factor and population as explanatory variables; effect of population: $F_{1,41}$<0.02, p>0.8). Yellowscore was not related to body condition in either population (Kissenda: r=-0.32, p=0.21, Makobe: r=-0.06, p=0.76).

Light transmission at Makobe and Kissenda Islands

The decline of light intensity with increasing depth was steeper at Kissenda Island than at Makobe (Figure 6.6): 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). With increasing depth, the ambient light spectrum shifted towards longer wavelengths, resulting in an increasing 'orange-ratio' (the light intensity in the 550-700 range divided by the intensity in the 400-550 range [Endler & Houde 1995]; Figure 6.6cd). The orange-ratio increased faster at Kissenda than at Makobe, yielding 0.86 and 0.75 for the two *P. nyererei* habitats respectively. Thus, the *P. nyererei* habitat at Kissenda is darker and more red-shifted than it is at Makobe (Figure 6.6e). Given that at Kissenda, it was too dark for spectrophotometry beyond 3 m depth, the actual differences between the two *P. nyererei* habitats may be even larger.

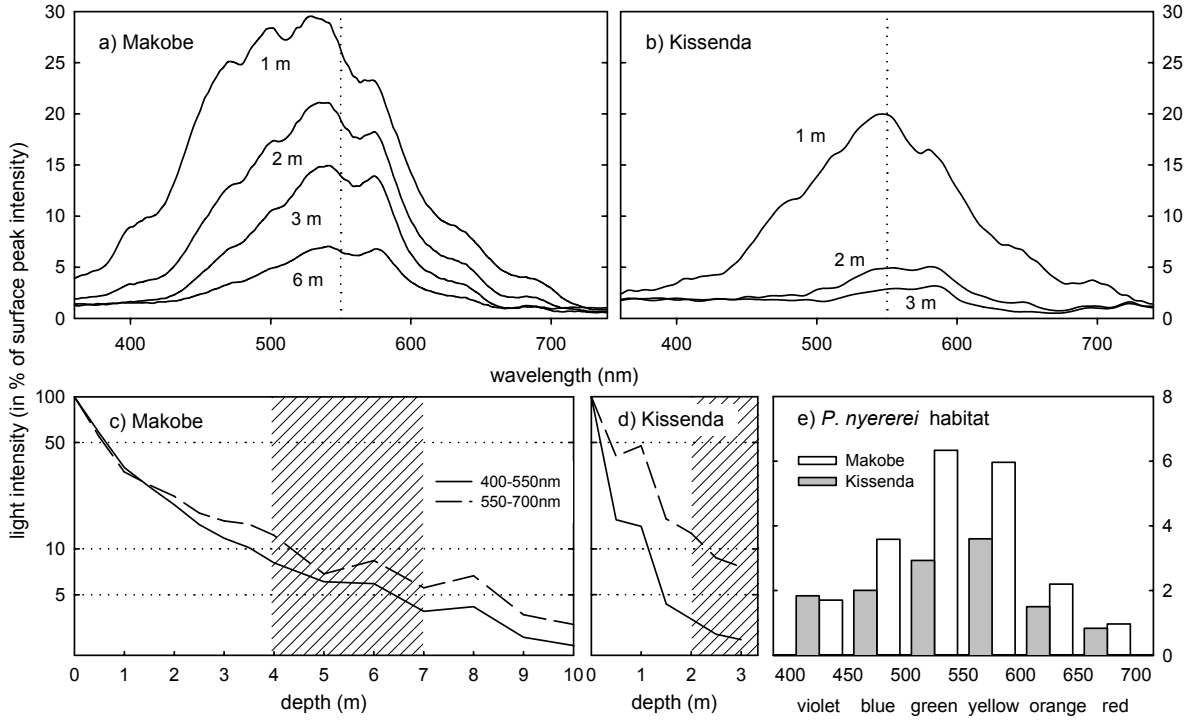


Figure 6.6 The photic habitat of *P. nyererei* at Makobe and Kissenda Islands. ab) Light spectra at different depths at Makobe and Kissenda. The dotted line indicates the 550 nm threshold, used for calculating the orange ratio. cd) Transmission of long (>550 nm, yellow-red) and short (<550, blue-green) wavelengths at both islands. Surface intensity is set at 100% for both wavelength classes. Axes are drawn to the same scale; shaded areas indicate *P. nyererei* habitat. e) Light intensity and spectral composition of the *P. nyererei* habitat at the two islands.

Discussion

Female *P. nyererei* mate choice behaviour differed significantly between Kissenda and Makobe populations. First, whereas females of both populations preferred redder males, this preference was significantly weaker in Kissenda than in Makobe females. Second, preferences were more strongly affected by male yellowscore in Kissenda than in Makobe females. Finally, Kissenda females preferred larger males but Makobe females did not. Females of the two populations did not differ in overall responsiveness to male courtship, overall choosiness or among-female consensus. Male coloration differed significantly between populations: Kissenda males had lower redscores and the hue of male red coloration was more orange-ish than in Makobe males.

These differences are probably associated with differences in light environment in the natural habitat of the fish. *P. nyererei* partly compensates for the low light transmission at Kissenda by inhabiting shallower water there than at Makobe. Also at other islands in south-eastern Lake Victoria, *P. nyererei* depth distribution varies with water transparency (including Makobe and Kissenda: $n=5$ islands, $r=0.96$, $p<0.01$; Figure 9.1). Despite this depth compensation however, the light environment of *P. nyererei* at Kissenda still differs from that at Makobe: it is darker and richer in long wavelengths. Therefore, the shift in male coloration from red towards yellow, both in body coverage and in hue, may be an adaptation to the ambient spectrum in this population: yellow and orange reflect more light than red and are better visible in a dark environment. Recent work indicates that also the visual system of *Pundamilia* has adapted to variation in the ambient light spectrum. Laboratory-bred F1 offspring from both *P. pundamilia* and *P. nyererei* from turbid water (Python Island, 10 km south-east of Kissenda, Secchi reading ~90 cm) were found to have more long-wavelength sensitive double cones than those from Makobe Island (Carleton et al. 2005).

Previous work (Chapter 3) has shown that the carotenoid composition of the red and yellow skin areas of male *P. nyererei* is very similar, and that the difference in hue is mainly due to differences in carotenoid content. This implies that the lower redscores and the more orange dorsal hue of Kissenda males correspond to a lower carotenoid content. Consequently, it could be argued that the population difference in male nuptial coloration is the result of a lower carotenoid availability at Kissenda Island, rather than an adaptive response to a different light environment. We think that this is unlikely for several reasons. First, the colour differences between Kissenda and Makobe males, as well as for other populations, persist in laboratory-raised F1 generations (Seehausen et al. 1997a; pers. obs.). Second, we have no indication that competition for food is more severe at Kissenda than at Makobe: *P. nyererei* population density (as well as total fish density) is lower (pers. obs.), and *P. nyererei* males are larger at Kissenda than at Makobe Island. Finally, the carotenoid-limitation mechanism yields predictions for female choice that are opposite to the findings presented here. As carotenoids become scarcer, carotenoid-based signals are more costly and thereby more informa-

tive (Andersson 1994; Grether 2000). Since *P. nyererei* red coloration is related to parasite load (Chapter 3), we would expect female preference for males with higher redscores to be stronger rather than weaker in carotenoid-limited environments. Parasite abundance and species composition may also differ between geographic locations. Likewise, variation in the strength and nature of predation pressure is likely to influence the expression of and (sexual) selection on male coloration (e.g. Endler 1983). Further studies must reveal how the relationships between *P. nyererei* male coloration, parasite infestation rates and female preferences vary with carotenoid availability, predation risk and water transparency.

In *P. nyererei* from Makobe Island, male coloration and behavioural courtship display are the most important choice criteria for females (Chapter 2). Therefore, the lower effectiveness of colour signalling in the turbid water of Kissenda Island could have selected for increased investment or elaboration of male courtship displays. We found the opposite of what would be expected according to such a mechanism: in the laboratory, Makobe males courted more frequently than Kissenda males. Similar differences exist between males from Makobe and males from the turbid waters of Python Island (Seehausen 1997). However, we found that Kissenda females preferred larger males whereas Makobe females did not, and that males were larger at Kissenda than at Makobe. Possibly, this reflects adaptation to an environment in which colour signalling is hampered.

We tested both populations in identical laboratory settings, with very clear water and an ambient light spectrum resembling daylight in shallow water. Our finding of significant population differences in female preferences under these conditions suggests that the observed correlation between male coloration and water transparency is not mediated by environmental variation alone. Rather, they indicate that female mating preferences have evolved in response to this variation. This implies that even when water transparency at Kissenda Island were to increase, male coloration would not evolve towards the condition at Makobe until female preferences would have adapted to the new signal transduction properties of the environment. This is the first evidence for intraspecific preference-trait coevolution in cichlid fish, confirming the earlier suggestion that the decrease in Lake Victoria water transparency affects both intraspecific sexual selection and interspecific mate choice (Seehausen et al. 1997a). The alternative hypothesis, that weaker female preferences are the result rather than cause of introgression of *P. pundamilia* genes, seems unlikely. This is because the variation in *Pundamilia* male nuptial coloration is clearly clustered in two distinct phenotypic classes, blue and red. Moreover, female preferences for these male types are probably fully divergent too. Species-assortative mating preferences have not been studied in this population, but females of the comparable population at Python Island show strong and consistent species-assortative mating preferences (Seehausen & Van Alphen 1998; Haesler & Seehausen 2005). These observations argue against strong admixture of species-specific genes for mating preferences and mating traits between *P. pundamilia* and *P. nyererei*.

Finally, the association between water transparency and *P. nyererei* depth distribution is likely to increase competition for food and space in shallow water. This mechanism may be of general importance in haplochromines, potentially causing additional extinctions through the reduction of ecological niche space. These threats underline the importance of measures to counteract 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 (Prof. Philip Bwathondi; Egid Katunzi) for hospitality and facilities. Mhoja Kayeba and Mohammed Haluna provided assistance in the field. We thank Hillary Mrosso, Peter Hiddinga, Roelof van der Kleij and Kees Hofker for their help with spectrophotometry, and Tom Van Dooren for helpful comments on the manuscript. The study was supported by the Netherlands Science Foundation (NWO-WOTRO 82-243) and a research grant from the Dobberke Foundation.