
Original articles

Ole Seehausen · Jacques J.M. van Alphen

The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei* complex)

Received: 11 April 1997 / Accepted after revision: 27 July 1997

Abstract We studied the effect of male coloration on interspecific female mate choice in two closely related species of haplochromine cichlids from Lake Victoria. The species differ primarily in male coloration. Males of one species are red, those of the other are blue. We recorded the behavioral responses of females to males of both species in paired male trials under white light and under monochromatic light, under which the interspecific differences in coloration were masked. Females of both species exhibited species-assortative mate choice when colour differences were visible, but chose non-assortatively when colour differences were masked by light conditions. Neither male behaviour nor overall female response frequencies differed between light treatments. That female preferences could be altered by manipulating the perceived colour pattern implies that the colour itself is used in interspecific mate choice, rather than other characters. Hence, male coloration in haplochromine cichlids does underlie sexual selection by direct mate choice, involving the capacity for individual assessment of potential mates by the female. Females of both species responded more frequently to blue males under monochromatic light. Blue males were larger and displayed more than red males. This implies a hierarchy of choice criteria. Females may use male display rates, size, or both when colour is unavailable. Where available, colour has gained dominance over other criteria. This may explain rapid speciation by sexual selection on male coloration, as proposed in a recent mathematical model.

Key words Cichlids · Lake Victoria · Mate choice · Sexual selection · Speciation

Introduction

Cichlids are among the most colourful and most species-rich families of fishes. The species flocks of the haplochromine clade that have evolved independently in many large lakes in East Africa are examples of explosive speciation, associated with traits strongly indicative of sexual selection. Haplochromine cichlids are polygynous, female mouth brooders without a pairbond and with strongly asymmetric parental investment. After spawning the brooding female refrains from feeding for a prolonged period, while males usually do not engage in broodcare but invest only gametes into the next generation. This life history makes haplochromines sensitive to effects of sexual selection by female mate choice. There is enormous interspecific diversity in male coloration and the cryptic females are assumed to recognize conspecific males by coloration (Ribbink 1986). As pointed out by Ryan and Rand (1993) sexual selection and species recognition are not antithetical. Female mate choice based on male colours may explain the great diversity of male coloration as well as the explosive speciation of haplochromine cichlids. Here we analyse the effect of male coloration on female mate choice in two closely related species by experimentally modifying male coloration.

Theory predicts that sexual selection is potentially a powerful agent of diversification. The predicted genetic correlation between mate preferences in the choosy sex (mostly the female) and preferred characters (ornaments) in the opposite sex, arising from assortative mating (Lande 1981), has been empirically demonstrated in several kind of fish (Houde and Endler 1990; Bakker 1993). Simultaneous selection for preferences and preferred traits drives character states away from the equilibria under natural selection (Fisher 1930; Lande 1981; Kirkpatrick and Ryan 1991) and, if of diversifying nature, can lead to the evolution of polymorphisms in preference and trait (Kodric-Brown and Nicoletto 1996), and may play a role in speciation (Lande 1981, 1982; Turner and Burrows 1995; Payne and Krakauer 1997).

O. Seehausen (✉) · J.J.M. van Alphen
Institute of Evolutionary and Ecological Sciences,
University of Leiden, P.O. Box 9516,
2300 RA Leiden, The Netherlands
Fax: +31-71-5274900;
e-mail: SEEHAUSEN@Rulsfb.LeidenUniv.NL

Characters indicative of the effects of sexual selection, such as sexual dimorphism and diverse male ornamentation (Dominey 1984; Coyne 1992), are well developed in the cichlid species flock of Lake Victoria (Greenwood 1974; Seehausen 1996a). At the same time, courtship behaviour of haplochromine cichlids is simple-compared to that of other cichlids (McElroy and Kornfield 1990). Though in many cichlids coloration is assumed to be important in direct female mate choice (e.g. Ribbink 1986), which requires discrimination between attributes of individual males (Wiley and Poston 1996), there are few experimental tests. Diverse male coloration could also have evolved by indirect choice which includes all other proximate mechanisms that restrict an individual's set of potential mates (Wiley and Poston 1996), by male-male competition for females (often referred to as intrasexual selection; Huxley 1938), or by a combination of these. Females could for instance have preferences for particular spawning locations rather than for particular males, as is known from wrasses, the closest marine relatives of cichlids (Warner 1987). Coloration could determine the outcome of interference competition between males over such locations. The available evidence does suggest that sexual selection on male traits in cichlid fish can be produced by both direct (Barlow et al. 1977, 1990; Barlow and Rogers 1978; Barlow 1983; Hert 1991) and indirect mate choice (McKaye et al. 1990).

Though assortative mating has been considered important in the maintenance (and presumably evolution) of reproductive isolation among the haplochromine cichlids of the African Great Lakes (Holzberg 1978; Marsh et al. 1981), it remained thus far unknown, whether it is based on male colours or on traits correlated with colour. In field studies it is almost impossible to disentangle the effects of colour and other traits, as well as direct versus indirect and female versus male choice. Assortative mating through direct female mate choice has been demonstrated for two closely related species of Lake Victoria cichlids (Seehausen 1997) but that study did not attempt to isolate possible effects of coloration and male courtship behaviour on female mate choice, which is the object of the present study.

The question has bearing upon whether sexual selection by direct female choice upon male coloration has played a role in the explosive speciation of cichlids in East Africa. If sexual selection on coloration through direct choice explains recent speciation events and the colour differences between sibling species, we would expect direct mate choice to result in reproductive isolation as long as the colour differences are visible. We addressed the problem experimentally by modifying male coloration (Long and Houde 1989; Milinski and Bakker 1990; Evans and Norris 1996), while excluding male-male interference competition. We tested with a pair of sibling species of Lake Victoria cichlids whether females choose actively and directly for males of their own species when (a) the interspecific differences in male coloration were visible and when (b) these differences were masked.

Methods

The species

Haplochromis nyererei (Witte-Maas and Witte 1985) and the undescribed *Haplochromis* "zebra nyererei" are two anatomically very similar forms that behave like biological species in some places and like colour morphs of one species in other places (Seehausen 1996, 1997). Both inhabit rocky shores and islands in Lake Victoria and live at many places sympatrically, usually exhibiting some ecological differences (Seehausen 1997; Seehausen and Bouton 1997). Males of both species have blackish underparts and blackish vertical bars on the flanks. The most conspicuous difference between them is coloration. Males of *H. nyererei* are bright crimson dorsally, yellow on the anterior flanks, and their dorsal fin is crimson. In the following we refer to them as red. Males of *H. "zebra nyererei"* are greyish white dorsally and on the flanks, and have a metallic blue dorsal fin. We will refer to them as blue. Females of both species have cryptic coloration with grey-brown vertical bars on a yellowish-grey ground colour, and yellowish transparent fins. Subtle differences in head anatomy help to distinguish the females of the two species. We refer to them as red and blue as well.

Courtship of both species resembles that of other haplochromine cichlid species (Carlstead 1981; McElroy and Kornfield 1990). A courtship bout begins either with male "approach", immediately followed by "lateral display", or directly with lateral display. In lateral display the male displays its body and fin coloration by posing in front of the female with an erected dorsal fin. If the female remains or approaches the male, the latter displays "quiver", a high-frequency shaking movement of the body during which the dorsal fin is partly folded, the anal fin stretched out, and the caudal fin bent away from the female. This is followed by "lead" swimming with waving tail beats towards the spawning pit. If the female follows the male to the pit, the male will present its egg dummies and start "circling" in the nest, which initiates spawning if the female joins in. Each of these actions is an integral part of a courtship behaviour chain. They are displayed in a rather consistent sequence (Carlstead 1981; McElroy and Kornfield 1990). For more details of courtship elements see Carlstead (1981) and McElroy and Kornfield (1990). Photographs and drawings of the elements are given by Seehausen (1996). Responsive females "approach" a displaying male and may "follow" it on its lead swimming, respectively "enter" and "over substrate" in McElroy and Kornfield (1990). If male coloration determines female choice, it is likely that it is assessed particularly during the lateral display posture [cf. McElroy and Kornfield (1990) who found indications that mate choice in Lake Malawi haplochromines is accomplished prior to onset of intense courtship].

Intermediate phenotypes were never found in most of the common geographical range of red and blue, although hybrids between red and blue can be obtained readily in the laboratory and are fully fertile. They were, however, caught in abundance at several places with exceptionally low water transparency, suggesting gene flow between the two forms at such places (Seehausen 1997). Individuals of two populations of each species, from regions, in which intermediate phenotypes had prior to 1996 not been observed (central Mwanza Gulf and Speke Gulf), were brought into the laboratory in 1992. F1, F2, and F3 generations were bred. Intermediate phenotypes never occurred. A first set of free mate choice experiments had confirmed that red and blue from both geographical regions mate assortatively under broad spectrum illumination (Seehausen 1997). For the present study we used red and blue from Python Islands (central Mwanza Gulf). This is a locality without intermediate phenotypes north of the turbid southern part of the Mwanza Gulf.

Housing conditions prior to the experiments

Males and females of each species were kept in separate aquaria, maintained at 24–26 °C, on a 12:12 h light:dark cycle. The aquaria

were illuminated with 40-W daylight fluorescent lightning. The fish were fed once a day a mix of ground shrimps and commercial flake food, once a day trout pellets, and several times per week red chironomid larvae.

Experimental design

Individual females were given free choice between a red and a blue male. Females moved freely in a 300-cm-long, 100-cm-deep and 60-cm-high (1800 l) experimental aquarium. Two six-sided enclosures from perspex, each measuring 45 cm in height and 60 cm diameter were placed into the experimental aquarium at its two short sides (Fig. 1). The bottom of the experimental aquarium was covered with a 10-cm-thick layer of sand into which the perspex enclosures were pressed. The water level in the experimental aquarium was kept just below the top of the perspex enclosures. The water temperature was maintained at 24–25 °C and filtered via a large out-board trickle filter with bio-balls. Each perspex enclosure was furnished with an air stone, providing oxygen and causing a slight overrun of water to maintain water exchange. Hence chemical communication between the female and the males was not excluded. One male was placed in each enclosure. In the enclosures the sand was piled up to a crater like structure with a pit in the centre, under an overhanging brick of 40 × 30 × 10 cm. Males accepted these artificial caves as the centre of their “territories” and made them the destination of their lead swimings during courtship.

Males had to be kept in enclosures to avoid interference competition between them which would be likely to influence female mate choice (Kodric-Brown 1993). To keep several males co-dominant, an aquarium needs to be richly structured, which makes observation difficult and introduces the possibility of female mate choice bias for criteria other than male traits. The purpose of constructing the male enclosures in a six-sided shape was to simplify the interpretation of female courtship behaviour by allowing females to follow males on their lead swimings in three dimensional space. The distance between enclosures, and thus the minimum distance between males was 2 m. Hence, females had abundant extraterritorial space to avoid visual contact with both males. To be courted, they had to move away from the centre of the aquarium, which was furnished with one hollow brick. A positive response to male courtship often consisted of a directed approach over a distance of more than 0.5 m. We consider such conditions an important prerequisite to reliably interpret female behaviour in cichlids without pair bond (see also McElroy and Kornfield 1990).

Colour manipulation

To manipulate female perception of male colour signals by illumination, we measured the coloration of those parts of the fish body that, to the human eye, differed most distinctly between the two species under daylight (dorsal fin and dorsum; Fig. 2A). Haplochromine cichlids possess trichromatic vision (van der Meer and Bowmaker 1995), and we assumed that perception of colour

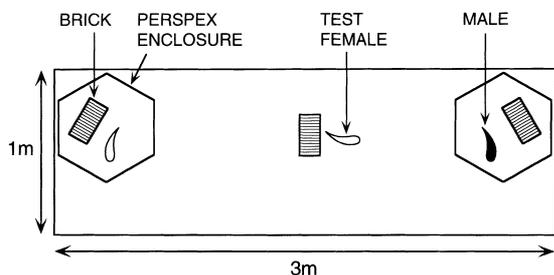


Fig. 1 Plan view of the experimental tank

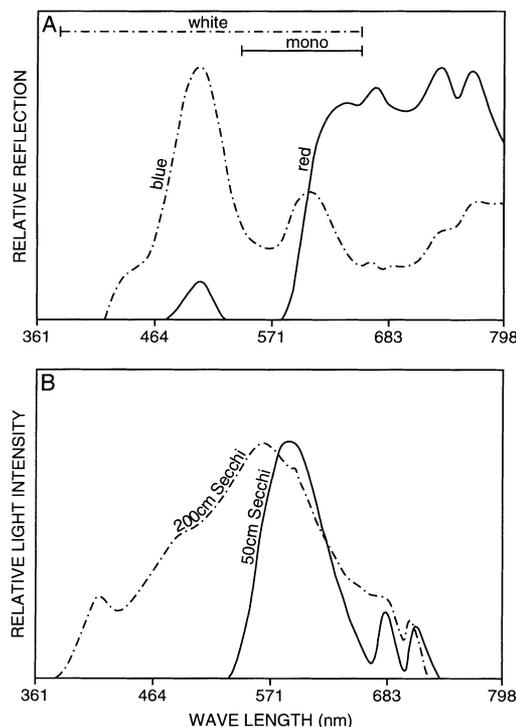


Fig. 2A,B Light spectra and male coloration. **A** The coloration of the spinous part of the dorsal fin in *Haplochromis nyererei* (red) and *H. “zebra nyererei”* (blue), in relation to the band widths of the light spectra used in white and in monochromatic light experiments. Fish coloration is measured as the relative reflected proportion of incidence light for each wave length. **B** Light spectra in 2 m depth in natural habitats of red and blue at a place with a Secchi transparency of 200 cm and a place with 50 cm. Spectra are drawn to the same height to demonstrate differences in colour. In voltage terms the peak light intensity at a Secchi transparency of 200 cm (595 V) was 7.6 times higher than at a transparency of 50 cm (78 V)

differences of the females in our experiment was similar to that of the human eye. The experimental aquarium was illuminated with four 40-W white fluorescent light tubes. In the first set of experiments the tubes were provided with a light filter, restricting the spectrum to long wave lengths (> 500 nm, double layer of filters, filter colour chrome orange, Lee Filters). The filter colour was chosen to mask the coloration differences between the two species. Blocking the blue light component in the incidence light, the filter caused blue parts of the fish body to appear greyish, and red parts to loose against an orange environment their conspicuousness (Fig. 2A). At the same time we simulated the spectral conditions that we measured in the lake at places with turbid waters, where intermediate phenotypes were abundant (Fig. 2B). We will refer to these light conditions as monochromatic light. Light intensity had to be considerably higher than in natural turbid water habitats, to make observation of the fishes possible.

In the second set of experiments the light filters were removed and illumination was broad spectrum. We will refer to this as white light. The distance between water surface and light tubes had to be increased in the white light experiments, to keep the intensity of illumination similar between the experiments. The white light spectrum resembled that found in the natural habitat at islands with clear water (Fig. 2B). Measurements of light spectra and fish coloration were taken with a computer-based microspectrometer (Ocean Optics PS 1000), operated with spectral data acquisition and processing software (Spectra Scope 2.2). Fish coloration was measured on slides as reflectance off the dorsal fin of sexually active fishes, photographed on Kodak Elite 100 ISO under daylight immediately after capture and with a grey unicolour card as reference.

Behaviour recording

Males were given a minimum of 4 h to acclimatise to the perspex enclosures before a trial was started. Behavioural interactions between the two males and one female in each trial were recorded over 60 min after the introduction of a female into the experimental aquarium. After that time the female was removed (in one experiment after only 45 min to prevent spawning at the perspex separation). Recorded elements of male courtship behaviour were lateral display (LD), quiver (QU) and lead (LE). Each courtship bout began with either an approach-lateral display sequence or directly with lateral display, and quantifying the frequency of encounters with lateral display was equivalent to quantifying the frequency of courtship bouts. Recorded elements of female courtship were approach (AP, upon male courtship) and follow (FO, upon male lead swimming). Data were recorded from behind a black curtain.

The frequency of female-male encounters with male courtship display (LD) was quantified as a proportion of all female-male encounters. The female's relative attractiveness to the two males offered in an experiment (difference score) was considered indicative of species specific effects on male courtship. The frequencies of female responses (AP, FO) to male LD were quantified as the proportions of encounters with LD followed by female responses out of all encounters with LD. The female's relative responsiveness to the two males offered in an experiment (difference score or preference score), was considered indicative of her preference. The null hypothesis for the white light treatment was that females would respond equally often to red and blue males. The null hypothesis for the monochromatic light treatment was that females would respond as they did under white light.

The subjects

The subjects were six adult naive F2 males (three red, three blue) and eight adult naive F3 females (four of each type). Standard length of each male was measured with a digital calliper to the nearest 0.5 mm. Weight of each male was measured in a known mass of water to the nearest 0.1 g on an electronic pan balance (Mettler). A condition factor was calculated from these measurements after Bolger and Connolly (1989) as $100 \times \text{weight (g)}/\text{length (cm)}^{2.76}$. Condition factors were 0.24, 0.24, 0.29 for the three red males, and 0.26, 0.31, 0.32 for the three blue males. Red and blue males were assigned to three pairs such that differences in condition factor within pairs were minimal.

For each trial one gravid female, determined by swelling of abdomen and urogenital opening (McElroy and Kornfield 1990; Balshine-Earn 1996), was selected. Females of the two forms were tested alternately in consecutive experiments. A male pair was tested with four females before it was exchanged, and was tested at most twice per day with an interval of at least 3 h. Females were only tested once per day. Each of the eight females had to choose once between each of the three male pairs in both light treatments. With one exception their level of responsiveness to male courtship did not change through the experiments (linear regressions of individual responsiveness on the sequence in which experiments were performed: $P > 0.1$). The exception was one blue female (female 6), the responsiveness of which decreased ($n = 6$, $r^2 = 0.74$, $P = 0.03$). On each male pair exchange, the positions of the male colour types in the experiment tank were exchanged. Experiments were completed within 2 months.

Statistics

For all tests the mean scores of individual females were used, each based on three trials with the three male pairs. The data distributions were tested for normality with the Kolmogorov test. Paired t -tests were used for pairwise comparison of the mean display rates of red and blue males to each female, and to test for species specific and overall effects of light treatment on display and response rates. Unpaired t -tests were used to test for differences in display and response rates between the species.

Mean difference scores of red minus blue males' proportions of display-encounters with a female, and of a female's responsiveness (proportion of display-encounters with female response) to red and blue males (preference scores) were then calculated. Trials in which a female did not respond to any male were omitted. To test for differences between females in their responsiveness to the two types of males, the mean preference scores of red and blue females were compared by unpaired t -tests. To test for an effect of relative male courtship frequencies on female mate choice, Pearson's product moment correlation coefficient was calculated between the difference scores for male display and female response frequencies. To test for differences in female mate preferences between the light treatments, paired t -tests were used. All t -tests were two-tailed, and all tests were performed with the Statgraphics Software package.

Results

Interspecific comparison of display and response rates

Female type had no species-specific effect on male courtship (Table 1A). Females were courted by blue males more frequently than by red males (white light: $n = 8$, $t = 3.49$, $P = 0.01$; monochromatic light: $n = 8$, $t = 3.05$, $P = 0.02$, paired t -tests). Light treatment had no effect on the rates of male display to a given female (red males: $n = 8$, $t = 0.47$, $P = 0.66$; blue males: $n = 8$, $t = 0.53$, $P = 0.61$, paired t -tests), but blue females under monochromatic light were courted more frequently than red females (means for red and for blue males separate $n_1 = 8$, $n_2 = 8$, $t = -2.82$, $P = 0.01$, unpaired t -test).

There was no difference in overall responsiveness between females of the two species (Table 1B; means over interactions with red and blue males in white light: $n_1 = 4$, $n_2 = 4$, $t = 0.37$, $P = 0.75$ for AP, $n_1 = 4$, $n_2 = 4$, $t = 1.03$, $P = 0.34$ for FO; in monochromatic light: $n_1 = 4$, $n_2 = 4$, $t = -0.63$, $P = 0.55$ for AP, $n_1 = 4$, $n_2 = 4$, $t = 0.57$, $P = 0.59$ for FO, unpaired t -tests). Nor was there an effect of light on the responsiveness of females to conspecific males expressed as approach ($n = 8$, $t = 0.06$, $P = 0.96$, paired t -test), but females tended to follow conspecific males under monochromatic light less often than under white light ($n = 8$, $t = 2.19$, $P = 0.07$, paired t -test).

The effect of male colour

Using the preference scores we found that under white light red females approached and followed red males more frequently than blue males, while blue females approached and followed blue males more frequently (Table 2, Fig. 3; $n_1 = 4$, $n_2 = 4$, $t = 4.32$, $P = 0.005$ for AP; $n_1 = 3$, $n_2 = 4$, $t = 3.49$, $P = 0.018$ for FO, unpaired t -tests).

Under monochromatic light, females tended to express weaker preferences (smaller preference scores: $n = 8$, $t = 1.84$, $P = 0.11$ for AP, $n = 6$, $t = 1.92$, $P = 0.11$ for FO, paired t -test), and there was no difference between the mate choice of the females of the

Table 1 A Mean proportion of encounters where male displayed to female in the two light treatments. Females 1 to 4 were red, females 5–8 were blue.

B Female response as the mean proportion of encounters where male display was followed by female approach (all means of $n = 3$ male pairs)

Female	Male type/light treatment			
	Red/white	Blue/white	Red/mono	Blue/mono
A				
1	0.20	0.39	0.53	0.61
2	0.58	0.76	0.40	0.37
3	0.14	0.41	0.42	0.57
4	0.74	0.70	0.52	0.57
\bar{x}_{1-4}	0.42	0.57	0.47	0.53
5	0.60	0.84	0.61	0.67
6	0.14	0.36	0.50	0.56
7	0.33	0.48	0.71	0.72
8	0.70	0.68	0.53	0.67
\bar{x}_{5-8}	0.44	0.59	0.59	0.66
\bar{x}_{1-8}	0.43	0.58	0.53	0.59
B				
1	0.11	0.05	0.17	0.15
2	0.11	0.05	0.17	0.18
3	0.06	0.02	0.00	0.13
4	0.41	0.07	0.17	0.25
\bar{x}_{1-4}	0.16	0.05	0.13	0.18
5	0.04	0.27	0.38	0.24
6	0.00	0.06	0.07	0.09
7	0.05	0.13	0.14	0.11
8	0.00	0.18	0.14	0.36
\bar{x}_{5-8}	0.02	0.16	0.18	0.20
\bar{x}_{1-8}	0.10	0.10	0.16	0.19

two species ($n_1 = 4$, $n_2 = 4$, $t = -0.73$, $P = 0.492$ for AP, $n_1 = 3$, $n_2 = 3$, $t = -0.90$, $P = 0.421$ for FO, unpaired t -tests), while, based on FO, females of both preferred blue males (based on AP: $n = 8$, $t = -1.22$, $P = 0.26$, based on FO: $n = 6$, $t = -3.79$, $P = 0.01$, paired t -tests). The difference between the light treatments in the degree of preference for conspecific males was significant (AP: $n = 8$, $t = 2.97$, $P = 0.021$; FO: $n = 6$, $t = 2.07$, $P = 0.093$, paired t -tests).

Female mate choice in white light was not correlated with relative male display rates (AP: $n = 8$, $r = 0.32$, $P = 0.44$; FO: $n = 7$, $r = 0.40$, $P = 0.39$). Since pairwise differences in male size were not affected by light treatment (the male pairs were the same under both light regimes), size differences cannot have determined mate choice under white light either. Under monochromatic light, female mate choice as expressed by approach tended to be correlated with relative male lateral

display frequencies (AP: $n = 8$, $r = 0.64$, $P = 0.09$; FO: $n = 6$, $r = -0.15$, $P = 0.78$).

Discussion

The experiments demonstrate that females that exhibited species-assortative mate choice when colour differences between males were visible, chose non-assortatively when these differences were masked by ambient light conditions. Male behaviour did not differ between light treatments, nor did overall female response rates. Though the small sample sizes caution against over-interpretation of the quantitative results, the following clearly emerged: (a) in monochromatic light, con- and heterospecific male courtship was a sufficient stimulus to elicit female response; (b) female preferences could be

Table 2 Difference scores of female responsiveness (AP approach, FO follow) to red and blue males (red male minus blue male) in the two light treatments. Females 1–4 were red, females 5–8 were blue (means of $[n]$ trials with different male pairs). Cases of no response to either male are omitted. In three combinations no FO was performed

Female	Behavioural event/light treatment			
	AP/white	FO/white	AP/mono	FO/mono
1	0.09 [2]	0.33 [1]	0.01 [3]	-0.08 [1]
2	0.09 [2]	0.23 [1]	-0.02 [2]	-0.20 [2]
3	0.06 [2]		-0.20 [2]	
4	0.34 [3]	0.74 [1]	-0.13 [2]	-0.08 [1]
\bar{x}_{1-4}	0.15	0.33	-0.08	-0.09
5	-0.35 [2]	-0.21 [2]	0.13 [3]	-0.04 [1]
6	-0.17 [1]	-0.08 [1]	-0.03 [2]	
7	-0.13 [2]	0.08 [1]	0.04 [3]	-0.04 [1]
8	-0.18 [3]	-0.15 [1]	-0.22 [3]	-0.14 [2]
\bar{x}_{5-8}	-0.21	-0.09	-0.02	0.06

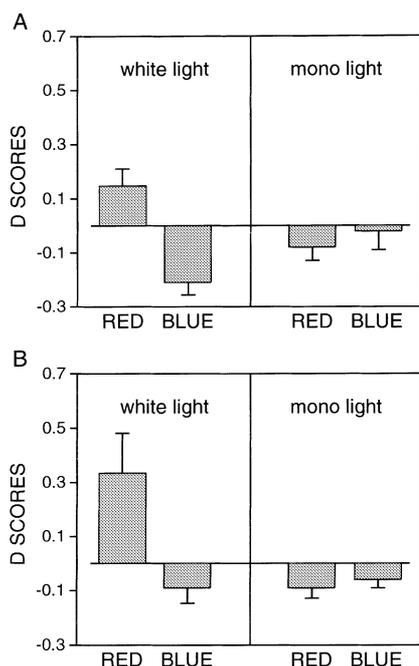


Fig. 3A,B Mean difference scores and standard error of female mate choice (responsiveness = proportion of encounters where male display was followed by female approach or follow to red male minus responsiveness to blue male) under white and monochromatic light: **A** approaching a male; **B** following a male. Each bar is based on the mean scores from four females that were tested each three times (*red* mate choice of red females, *blue* mate choice of blue females)

altered by manipulating the perceived male coloration implying that the colour itself, rather than possible correlated characters, is used in interspecific mate choice. The break-down of assortative mating under monochromatic light is most likely to be explained by the masking of visual signals. Analogous results were obtained by Long and Houde (1989) on intraspecific female mate choice in guppies (*Poecilia reticulata*). Female mate preferences for the brighter orange males broke down when ambient light conditions reduced the conspicuousness of the orange.

If relative female mate preferences reflect relative gene flow, then the isolation between the two species used in our experiments should in nature depend on the light conditions in their habitats. One may expect that criteria for mate-preferences other than colour evolve (and possibly mechanisms of reproductive isolation), where hue of coloration is (secondarily) becoming ineffective in interspecific mate choice. It is unknown how much time is needed to evolve such signals, and changes in the environment may take place more quickly. Circumstantial evidence suggests that the extent of gene flow between the sibling species used in this study indeed depends on light conditions in nature (Seehausen 1997).

The results have implications for the understanding of the role of sexual selection in the evolution of male coloration in cichlids. In our study, female mate choice was directly determined by male coloration. Though the possibility that sexual selection through indirect mate

choice is also acting on male coloration is of course not ruled out by our results, male coloration does underlie selection through direct choice (*sensu* Wiley and Poston 1996), involving the capacity for individual assessment of potential mates by the female.

Direct mate choice is in agreement with studies that had previously shown the capacity for individual assessment of mates based on other traits in other cichlids (Noble and Curtis 1939; Barlow et al. 1977, 1990; Barlow and Rogers 1978; Hert 1991; Rogers and Barlow 1991; Balshine-Earn 1996). This is a prerequisite for females to use male traits as an indicator of male quality as the male handicap hypothesis suggests (Zahavi 1975; Maynard Smith 1976; Hamilton and Zuk 1982; Pomiankowski 1987; Kennedy et al. 1987; Grafen 1990; reviewed by Collins 1993).

That females of both species in our study under monochromatic light exhibited 'follow' more frequently to blue males may indicate that females use male display rates, size, or both as choice criteria when colour is unavailable. This is in concordance with results obtained in other studies on mate choice in fish. Size is known to be important in many species (cichlids: Barlow et al. 1977; Barlow 1983; Rogers and Barlow 1991; Balshine-Earn 1996; other fishes: Borowsky 1981; Reynolds and Gross 1992; Endler and Houde 1995), and display rates have been reported to contribute to female mate choice decisions in guppies (Endler 1983; Kennedy et al. 1987; Kodric-Brown 1989, 1993). The relative contributions of male size, display rate and coloration to intraspecific female choice decisions have sometimes been difficult to disentangle (e.g. Barlow et al. 1977, 1990; Barlow and Rogers 1978; Barlow 1983; Farr 1980; Reynolds and Gross 1992; Kodric-Brown and Nicoletto 1996 versus Kodric-Brown 1985; Long and Houde 1989). Our experiments show that different results in this respect may at least partly be due to the circumstance that the relative importance of different male traits may change with environmental parameters, and hence with experimental design (Barlow 1983; Kodric-Brown 1989, 1993; Endler and Houde 1995; Hunt et al. in press).

In the case reported here of mate choice between sibling species, colour is dominant over display rate and size. The implicit hierarchy of choice criteria in intra- and interspecific situations, and the impact of the environment on it, can be tested experimentally and by tracing environmental correlates of male trait expression in nature. That the red females in our study did not identify red males on other possible species specific characters when colour was unavailable, indicates that speciation, while occurring with divergence in male coloration, was accompanied by little if any divergence in other traits relevant to direct mate choice. If females simply choose for colour within a certain range of male body shape, speciation could occur if quantitative changes in the physiology of the signal receptor in some females cause a branch point in the evolution of the male signal.

Observations on the distribution of male coloration over sibling species pairs of Lake Victoria cichlids

suggest that blue versus red or yellow is a very common colour dichotomy among closely related species (Hoogerhoud et al. 1983; Seehausen 1996a p. 273; Seehausen and Bouton 1996). The mechanism underlying the evolution of this signal dichotomy is unknown but physiological differences among individual females, causing different colour preference due to different sensory bias may be an explanation (Basolo 1990; Ryan and Keddy-Hector 1992). Polymorphism in colour vision is known for instance in guppies (Basolo and Endler 1995).

Our experiments reveal a preference for conspecific signals in simultaneous choice but response also to non-conspecific signals in a no-choice situation. This indicates that females did not follow a simple absolute preference rule (Real 1990). When the female capacity to assess male colour differences was neutralized by masking coloration differences between males, females responded to males of both species without a decrease in the level of overall responsiveness. This keeps the costs low that direct choice is thought to incur, such as lost opportunities, loss of time and energy (Wiley and Poston 1996).

The type of mate-preference rule is important to understanding the potential role of sexual selection in speciation. Sibling species pairs that differ from each other predominantly in male coloration and contrasting female colour preferences, and in which females follow the “best of n rule” in mate selection, possess the basic qualities required for a recently suggested model of sympatric speciation by sexual selection (Turner and Burrows 1995). Polymorphism in male coloration, maintained by polymorphism in female choice has been reported for guppies (Kodric-Brown 1993; Kodric-Brown and Nicoletto 1996). Female preference polymorphisms have also been found in anurans (Gerhardt 1991) and insects (Wagner et al. 1995).

The male trait in the speciation model of Turner and Burrows (1995) was male colour shade, ranging from light to dark. Measurements of the reflection spectra show that the difference in hue among the sibling species in our study is relative (red/blue ratio; Fig. 2a). Thus, it is quantitative and may be selected for like the light/dark difference in the model. To test whether our results match the assumptions of the model, it is important to examine the intraspecific female preference function (cf. Ryan and Rand 1993) and the genetic structure underlying male coloration and female colour preferences.

The species flock of haplochromine cichlids in Lake Victoria consisted of more than 500 species until human intervention caused the extinction of many (Barel et al. 1985; Witte et al. 1992). Palaeolimnological and palynological work showed that Lake Victoria had entirely dried up and filled up again as recently as 12,400 years ago, at the end of the last glacial period, setting a very narrow time frame for the evolution of its cichlids (Johnson et al. 1996). The strong preference for conspecific male coloration that we observed in females of two, otherwise very similar, haplochromine species, suggests that direct mate choice based on coloration has been important in speciation. While the ecological diversity of Lake Victoria

cichlids appears largely due to a flexible pharyngeal jaw apparatus which enables rapid evolutionary diversification (Galis and Drucker 1996), sexual selection on coloration could account the existence of many ecologically very similar species on most adaptive levels. The breakdown of preferences for conspecific mates in monochromatic light conditions, such as are found in turbid water, is a warning to those concerned about the conservation of Africa's unique cichlid fish fauna.

Acknowledgements We thank the Tanzanian Commission for Science and Technology for the research permit for OS, the Tanzanian Fisheries Research Institute (Prof. P.O.J. Bwathondi, E.F.B. Katunzi) for hospitality, and the Fisheries Division for the permit to export live fishes; Claus Schaefer, Ludwig Schadhauer, Radhmina Kiteri, Hosea Gonza Mbilinyi, Hellen Nielsen, Anna Samwel-Terry and Laurent Assembe Tsongui for logistical support; Sarah Collins for advice on data analysis; Sarah Collins, Hans Slabekoorn, Frietson Galis, George Turner, Niels Bouton, Frans Witte, and one anonymous referee for insightful comments on the manuscript. The study was supported by a research grant from TETRA and the German Cichlid Association (DCG).

References

- Bakker TCM (1993) Positive genetic correlation between female preference and preferred male ornament in sticklebacks. *Nature* 363:255–257
- Balshine-Earn S (1996) Reproductive rates, operational sex ratios and mate choice in St. Peter's fish. *Behav Ecol Sociobiol* 39:107–116
- Barel CDN, Dorit P, Greenwood PH, Fryer G, Hughes N, Jackson PBN, Kawanabe H, Lowe-McConnell RH, Nagoshi M, Ribbink AJR, Trewavas E, Witte F, Yamaoka K (1985) Destruction of fisheries in Africa's lakes. *Nature* 315:19–20
- Barlow GW (1983) The benefits of being gold: behavioral consequences of polychromatism in the midas cichlid, *Cichlasoma citrinellum*. *Environ Biol Fish* 8:235–247
- Barlow GW, Rogers W (1978) Female midas cichlids' choice of mate in relation to parents' and to own color. *Biol Behav* 3:137–145
- Barlow GW, Rogers W, Capetto RV (1977) Incompatibility and assortative mating in the midas cichlid. *Behav Ecol Sociobiol* 2:49–59
- Barlow GW, Francis RC, Baumgartner JV (1990) Do the colors of parents, companions and self influence assortative mating in the polychromatic midas cichlid? *Anim Behav* 40:713–722
- Basolo AL (1990) Male swords and female preferences. *Science* 253:1426–1427
- Basolo AL, Endler JA (1995) Sensory biases and the evolution of sensory systems. *Trends Ecol Evol* 10:489
- Bolger T, Connolly PL (1989) The selection of suitable indices for the measurement and analysis of fish condition. *J Fish Biol* 34:171–182
- Borowsky RL (1981) Tailspots of *Xiphophorus* and the evolution of conspicuous polymorphisms. *Evolution* 35:345–358
- Carlstead K (1981) Motivational influences on individual and species differences in responses to conspecifics in three *Haplochromis* species (Pisces: Cichlidae). Thesis, University of Groningen
- Collins S (1993) Is there only one type of male handicap? *Proc R Soc Lond B* 252:193–197
- Coyne JA (1992) Genetics and speciation. *Nature* 355:511–515
- Dominey WJ (1984) Effects of sexual selection and life history on speciation: species flocks in African cichlids and Hawaiian *Drosophila*. In: Echelle AA, Kornfield I (eds) *Evolution of fish species flocks*. University of Maine Press, Orono, pp 231–249

- Endler JA (1983) Natural and sexual selection on color patterns in Poeciliid fishes. *Environ Biol Fish* 9:173–190
- Endler JA, Houde AE (1995) Geographical variation in female preferences for male traits in *Poecilia reticulata*. *Evolution* 49:456–468
- Evans MR, Norris K (1996) The importance of carotenoids in signaling during aggressive interactions between male firemouth cichlids (*Cichlasoma meeki*). *Behav Ecol* 7:1–6
- Farr JA (1980) Social behavior patterns as determinants of reproductive success in the guppy *Poecilia reticulata* Peters (Pisces: Poeciliidae): an experimental study of the effects of intermale competition, female choice, and sexual selection. *Behaviour* 74:38–91
- Fisher RA (1930) The genetical theory of natural selection. Clarendon, Oxford
- Galis F, Drucher EG (1996) Pharyngeal biting mechanisms in centrarchid and cichlid fishes: insight into a key evolutionary innovation. *J Evol Biol* 9:641–670
- Gerhardt HC (1991) Female mate choice in treefrogs: static dynamic acoustic criteria. *Anim Behav* 42:615–635
- Grafen A (1990) Biological signals as handicaps. *J Theor Biol* 144:517–546
- Greenwood PH (1974) Cichlid fishes of Lake Victoria, east Africa: the biology and evolution of a species flock. *Bull Br Mus Nat Hist Zool Suppl* 6:1–134
- Hamilton WD, Zuk M (1982) Heritable true fitness and bright birds: a role for parasites. *Science* 218:384–387
- Hert E (1991) Female choice based on egg-spots in *Pseudotropheus aurora* Burgess 1976, a rock-dwelling cichlid of Lake Malawi, Africa. *J Fish Biol* 38:951–953
- Holzberg S (1978) A field and laboratory study of the behaviour and ecology of *Pseudotropheus zebra* (Boulenger), an endemic cichlid of Lake Malawi (Pisces: Cichlidae). *Z Zool Syst Evol Forsch* 16:171–187
- Hoogerhoud RJC, Witte F, Barel CDN (1983) The ecological differentiation of two closely resembling *Haplochromis* species from Lake Victoria (*H. iris* and *H. hiatus*; Pisces, Cichlidae). *Neth J Zool* 33:283–305
- Houde A, Endler JA (1990) Correlated evolution of female mating preferences and male color patterns in the guppy *Poecilia reticulata*. *Science* 248:1405–1408
- Hunt S, Cuthill IC, Swaddle JP, Bennett ATD (in press) Ultraviolet vision and band colour preferences in female zebra finches, *Taeniopygia guttata*. *Anim Behav*
- Huxley JS (1938) Darwin's theory of sexual selection and the data subsumed by it, in the light of recent research. *Am Nat* 72:416–433
- Johnson TC, Scholz CA, Talbot MR, Kelts K, Ricketts RD, Ngobi G, Beuning K, Ssemmanda I, McGill JW (1996) Late Pleistocene desiccation of Lake Victoria and rapid evolution of cichlid fishes. *Science* 273:1091–1093
- Kennedy CEJ, Endler JA, Poyton SL, McMinn H (1987) Parasite load predicts mate choice in guppies. *Behav Ecol Sociobiol* 21:291–295
- Kirkpatrick M, Ryan MJ (1991) The evolution of mating preferences and the paradox of the lek. *Nature* 350:33–38
- Kodric-Brown A (1985) Female preference and sexual selection for male coloration in the guppy (*Poecilia reticulata*). *Behav Ecol Sociobiol* 17:199–206
- Kodric-Brown A (1989) Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. *Behav Ecol Sociobiol* 25:393–401
- Kodric-Brown A (1993) Female choice of multiple male criteria in guppies: interacting effects of dominance, coloration and courtship. *Behav Ecol Sociobiol* 32:415–420
- Kodric-Brown A, Nicoletto PF (1996) Consensus among females in their choice of males in the guppy *Poecilia reticulata*. *Behav Ecol Sociobiol* 39:395–400
- Lande R (1981) Models of speciation by sexual selection on polygenic traits. *Proc Natl Acad Sci USA* 78:3721–3725
- Lande R (1982) Rapid origin of sexual isolation and character divergence in a cline. *Evolution* 36:213–223
- Long KD, Houde AE (1989) Orange spots as a visual cue for female mate choice in the guppy (*Poecilia reticulata*). *Ethology* 82:316–324
- McElroy DM, Kornfield I (1990) Sexual selection, reproductive behavior, and speciation in the mbuna species flock of Lake Malawi (Pisces: Cichlidae). *Environ Biol Fish* 28:285–294
- McKaye KR, Louda SM, Stauffer JR Jr. (1990) Bower size and male reproductive success in a cichlid fish lek. *Am Nat* 135:597–613
- Marsh AC, Ribbink AJ, Marsh BA (1981) Sibling species complexes in sympatric populations of *Petrotilapia* Trewavas (Cichlidae, Lake Malawi). *Zool J Linn Soc* 71:253–264
- Maynard Smith J (1976) Sexual selection and the handicap principle. *J Theor Biol* 57:239–242
- Meer HJ van der, Bowmaker JK (1995) Interspecific variation of photoreceptors in four co-existing haplochromine cichlid fishes. *Brain Behav Evol* 45:232–240
- Milinski M, Bakker TCM (1990) Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature* 344:330–333
- Noble GK, Curtis B (1939) The social behavior of the jewel fish, *Hemichromis bimaculatus* Gill. *Bull Am Mus Nat Hist* 76:1–46
- Payne RJH, Krakauer DC (1997) Sexual selection, space, and speciation. *Evolution* 51:1–9
- Pomiankowski AN (1987) Sexual selection: the handicap principle does work – sometimes. *Proc R Soc Lond B* 231:123–145
- Real L (1990) Search theory and mate choice. I. Models of single-sex discrimination. *Am Nat* 136:376–404
- Reynolds JD, Gross MR (1992) Female mate preference enhances offspring growth and reproduction in a fish, *Poecilia reticulata*. *Proc R Soc Lond B* 250:57–62
- Ribbink AJ (1986) The species concept, sibling species and speciation. *Ann Mus Roy Afr Centre Sci Zool* 251:109–116
- Rogers W, Barlow GW (1991) Sex differences in mate choice in a monogamous biparental fish, the midas cichlid (*Cichlasoma citrinellum*). *Ethology* 87:249–261
- Ryan MJ, Keddy-Hector A (1992) Directional patterns of female mate choice and the role of sensory biases. *Am Nat* 139:s4–s35
- Ryan MJ, Rand AS (1993) Species recognition and sexual selection as a unitary problem in animal communication. *Evolution* 47:647–657
- Seehausen O (1996) Lake Victoria Rock Cichlids, taxonomy, ecology, and distribution. *Verduijn, Zevenhuizen*
- Seehausen O (1997) Distribution of, and reproductive isolation among, colour morphs of a rock-dwelling Lake Victoria cichlid (*Haplochromis nyererei*). *Ecol Freshwater Fish* 6:59–66
- Seehausen O, Bouton N (1996) Polychromatism in rock dwelling Lake Victoria cichlids: types, distribution, and observations on their genetics. *Cichlids Yearb* 6:36–45
- Seehausen O, Bouton N (1997) Microdistribution and fluctuations in niche overlap in a rocky shore cichlid community in Lake Victoria. *Ecol Freshwater Fish* 6:161–173
- Turner GF, Burrows MT (1995) A model of sympatric speciation by sexual selection. *Proc R Soc Lond B* 260:287–292
- Wagner WE Jr, Murray AM, Cade WH (1995) Phenotypic variation in the mating preferences of female field crickets, *Gryllus integer*. *Anim Behav* 49:1269–1281
- Warner RR (1987) Female choice of sites versus mates in a coral reef fish, *Thalassoma bifasciatum*. *Anim Behav* 35:1470–1478
- Wiley RH, Poston J (1996) Indirect mate choice, competition for mates, and coevolution of the sexes. *Evolution* 50:1371–1381
- Witte F, Goldschmidt T, Wanink J, Oijen M van, Goudswaard K, Witte-Maas E, Bouton N (1992) The destruction of an endemic species flock: quantitative data on the decline of the haplochromine cichlids of Lake Victoria. *Environ Biol Fish* 34:1–28
- Witte-Maas E, Witte F (1985) *Haplochromis nyererei*, a new cichlid fish from Lake Victoria named in honour of Mwalimu Julius Nyerere, president of Tanzania. Brill, Leiden
- Zahavi A (1975) Mate selection – a selection of a handicap. *J Theor Biol* 53:205–214