Cichlid Fish Diversity Threatened by Eutrophication That Curbs Sexual Selection

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Cichlid fish species of Lake Victoria can interbreed without loss of fertility but are sexually isolated by mate choice. Mate choice is determined on the basis of coloration, and strong assortative mating can quickly lead to sexual isolation of color morphs. Dull fish coloration, few color morphs, and low species diversity are found in areas that have become turbid as a result of recent eutrophication. By constraining color vision, turbidity interferes with mate choice, relaxes sexual selection, and blocks the mechanism of reproductive isolation. In this way, human activities that increase turbidity destroy both the mechanism of diversification and that which maintains diversity.

In the Great Lakes of Africa, large and diverse species flocks of cichlid fish have evolved rapidly (*1*, *2*). Lake Victoria, the largest of these lakes, had until recently at least 500 species of haplochromine cichlids (*3*). They were ecologically so diverse that they utilized almost all resources available to freshwater fishes in general (*2*), despite having evolved in perhaps as little as 12,400 years (*1*) and from a single ancestral species (*4*). This species flock is the most notable example of vertebrate explosive evolution known today. Many of its species have vanished within two decades (*5*, *6*), which can only partly be explained by predation by the introduced Nile perch (*Lates* spp.). Stenotopic rock-dwelling cichlids, of which there are more than 200 species (*7*), are rarely eaten by Nile perch (*8*). Yet, many such species have disappeared in the past 10 years (*6*, *7*). Because gene flow between island populations of these cichlids is effectively limited by stretches of sand and mud bottom (*9*), populations underlie local selection regimes. Here we demonstrate that increasing turbidity, by curbing the impact of sexual selection on sexual isolation, is responsible for the decline in cichlid diversity.

The seven Great Lake basins of tropical Africa in which haplochromine cichlids formed endemic species flocks have distinctly clearer waters than the five in which they did not (300 to 2200 versus 20 to 130 cm maximum Secchi disc readings; $n = 12$, $t = 2.99$, $P = 0.015$). Significance increases when the three very large lakes Victoria, Malawi, and Tanganyika are excluded ($n = 9$, $t = 3.83$, $P = 0.009$), ruling out the alternative hypothesis that lake size explains the difference. Lake Victoria has rapidly eutrophied (*10*) and become turbid. Water transparency decreased in deep open waters from 5.5 to 8 m in the 1920s to 1.3 to 3 m in the 1990s and decreased in the littoral zone from 3 to 1.5 m within the past decade (*11*). We have investigated the effect of these changes on the cichlids. Postmating reproductive barriers have not been found among Lake Victoria cichlids (*12*), presumably because of their phylogenetic youth. Reproductive isolation among sympatric species is maintained only by mate choice. Haplochromines have vibrantly colored males and usually cryptically colored females (*2*, *7*). Their eyes are equipped with three retinal cone pigments that cover the light spectrum from blue to red (*13*).

Sympatrically living, closely related species usually have male coloration at opposite ends of the color spectrum, one being blue whereas the other one is red or yellow (*14*). Strikingly, the same dichotomy is found among conspecific male color morphs (*14*) and matches the two absorbance peaks of the retinal pigments (*13*). If females prefer conspicuous over nonconspicuous males (*15*), individual variation in color vision could be responsible for the origin of the observed dichotomy in male coloration, and interspecific variation in color vision could be responsible for its maintenance (*16*, *17*).

We examined the role of color in mate choice of Lake Victoria cichlids in laboratory experiments. Females of a sympatric red/blue sibling species pair (*Haplochromis nyererei*/"zebra nyererei") preferred conspecific over heterospecific males under broad-spectrum illumination ($n = 8$, $t = 3.43$, $P = 0.01$; paired two-tailed t test), but mated indiscriminately under monochromatic light where color differences were masked ($n = 8$, $t = -0.35$, $P = 0.74$) (*18*). Mate preferences of two sympatric color morphs of *Neochromis* "blue scraper" were positively assortative (*19*). Hence, haplochromine cichlids choose mates within and across species on the basis of coloration and in doing so distinguish between mates of their own and other species and color morphs. Light conditions constrain this choice. Eutrophication causes a decrease in light penetration and a narrowing of the light spectrum due to strong loss of shortwave light (*20*). Therefore, it must pose constraints on

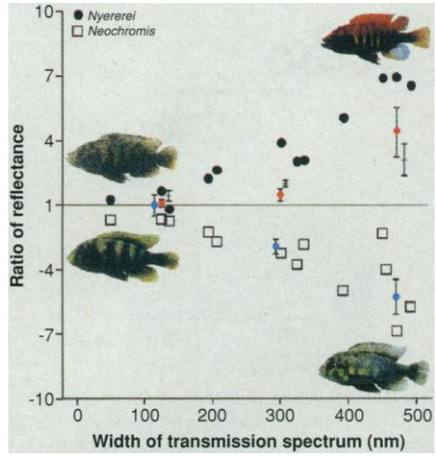

Fig. 1. Hue of body colors as a function of light transmission. The redness of male nuptial coloration of *H. nyererei* and the blueness of *Neochromis* "velvet black"/"blue scraper" in 13 populations along the south-north transect is quantified as the ratio of reflectance 610 nm/515 nm and 515 nm/610 nm, respectively, at each island (*H. nyererei* was absent from one). The horizontal line represents equal reflectance of red (610 nm) and blue (515 nm). Above the line, red is more reflected than blue; below it, blue is more reflected than red (negative ratio on the y axis). Three kinds of data are presented: (i) The brightest males (filled circles and open squares). Brightness (relative to population average) indicates sexual activity (Spearman rank correlation coefficient between brightness and gonadal development within a population of *H. nyererei*, both scored on a 5-point scale: $r = 0.67$, $P < 0.00001$, $n = 42$). (ii) Population means ± SE for two extreme and one intermediate population of each species. They were obtained by measuring (from left to right) 5, 5, 7 individuals of *H. nyererei* (red points) and 6, 3, 5 of *Neochromis* (blue points), covering the range of variation of sexually active males in each population. (iii) Means ± SE for laboratory-bred stocks of the same three populations of *H. nyererei* (bars). Populations were spawned and raised separately under standardized conditions. After the fishes had attained maturity, they were kept in three groups of 10 males. Of each group, the five most intensely colored ones that became sexually active were eventually photographed for measurements (*31*). The difference (y) in hue between wild males of the red and the blue species is related to spectral bandwidth (x) as $y = -1.83 + 0.02x$, $r^2 = 0.88$, $F = 71.54$, $P < 0.00001$. The difference between the mean values for wild males from dull and colorful populations is in both species significant (*H. nyererei*: $Z = 1.95$, $P = 0.05$; *Neochromis*: $Z = 2.65$, $P < 0.01$) and is significant between the captive-bred fishes as well ($Z = 2.10$, $P < 0.05$). The larger standard errors in more colorful populations reflect the larger color diversity found in clear-water populations.

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the functional diversity of color signals. It should limit the number of species that can be sexually isolated and the number of color morphs that are maintained merely by preferences for different color signals.

We studied the effect of light on cichlid color and diversity on a south-north transect of 13 rocky islands in southern Lake Victoria (70 km across Mwanza and Speke Gulf). We measured physical and biotic variables, including light, and spectral composition of male nuptial coloration in a red and a blue species of rock-dwelling haplochromines, *H. nyererei* and *Neochromis* "velvet black," including its offshore replacement *Neochromis* "blue scraper" (*7*). We determined color morph diversity in *Neochromis* "velvet black," overall species diversity of rock-dwelling haplochromines, and diversity within the most speciose genera (*Neochromis*, *Nyererei* complex, *Paralabidochromis*).

The largest part of interpopulation variation in hue of male nuptial coloration was explained by the aquatic light regime (Table 1 and Fig. 1). Male coloration is more distinctly red or blue where visual conditions enhance the effect of red and blue color signals: namely, in clear, broad spectrum–illuminated water that contains sufficiently red and blue downwelling light to make a contrast against the yellowish sidewelling light. Breeding three populations of *H. nyererei* in aquaria for one to three generations demonstrated that the differences in hue among populations are heritable (Fig. 1). This experiment rules out the alternative hypothesis that dull coloration is induced by correlated phenotypic effects of turbidity such as lack of carotenoids in the diet.

Cichlids are more easily spotted by their main predators, visually hunting birds (cormorants and egrets) and otters, in clear waters than in turbid waters. Cormorants catch predominantly brightly colored cichlids (*21*), and piscivorous birds tended to be more abundant where the water was clear ($y = 0.05 + 0.0057x$, $F = 3.55$, $r^2 = 0.24$, $P = 0.086$). A natural selection hypothesis, therefore, would make the prediction that is opposed to the observations, that less bright fish occur in more transparent water. Hence, male coloration is most likely determined by sexual selection (*22*, *23*). Directional sexual selection for conspicuously red or blue males, however, can exist only where such colors are visible. The drab colors of males in turbid water indicate that male coloration is costly and is lost when color visibility decreases (*17*, *23–25*).

Aquatic light conditions also explained most of the variation in intraspecific color morph diversity and in species number (Table 1). The clearer the water and the broader the spectrum of transmitted light, the more color morphs of a species (Fig. 2B) and the more species of a genus (Figs. 2 and 3) coexist at a site. We obtained similar results on a 53-km-long east-west transect of nine rocky habitat islands that was measured in 1996 only, along which the gradients of water transparency and light trans-

Table 1. Minimum adequate regression models required to explain variation in cichlid coloration, color morph, and species diversity. Fourteen environmental variables were measured: habitat patch size, distance from the mainland, maximum water depth, mean size of rock boulders and its variation, mean steepness of shore slope and its variation, resource abundance (measured as cichlid fish abundance), predator abundance: Nile perch and piscivorous birds, water transparency, bandwidth of the transmission light spectrum, transmission of 400 nm light, and the ratio of transmission 580 nm by 400 nm. Of the last four, being different measures of the light environment, only one, which correlated highest with the dependent variable, was included in the regression model design procedure. Therefore, 11 environmental variables plus sampling effort were included. Cichlid diversity, resource abundance, and abundance of Nile perch and birds were determined over three 3-month periods spread over 3 years, water transparency and light transmission of the water were measured twice with an interval of 1 year. Fish coloration and light transmission were measured and quantified as described in (*31*) and Fig. 1. Regression models were calculated by a stepwise variable-selection procedure (*32*) with forward selection and a minimum F value of 4 for variables to enter the model. Significance levels: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Dependent variable	Explanatory variables (and the sign of correlation)	r^2	F	P-value model	r^2 model
	Coloration				
Redness of *H. nyererei*	Width of tranmission spectrum (+)	0.89	161.94****	<0.0001	0.95
	Bird density (+)	0.04	10.64*		
	Mean rock size (+)	0.03	5.74		
Blueness of VBL*	Water transparency (+)	0.65	35.32***	0.0001	0.86
	Maximum depth† (+)	0.17	9.71*		
	Shore slope (−)	0.04	4.01		
	Color morph diversity				
VBL color morph number*	$T_{400\ nm}$‡ (+)	0.85	59.05***	<0.0001	0.99
	Maximum depth† (+)	0.10	42.09***		
	Distance to the mainland (+)	0.03	30.96***		
	Area size (+)	0.009	10.02*		
	Nile perch abundance (−)	0.006	10.70*		
Shannon-Wiener index of VBL morph diversity*	$T_{400\ nm}$‡ (+)	0.79	80.99****	<0.0001	0.91
	Variation in boulder size (+)	0.12	17.25**		
	Species diversity				
Total species number	Width of transmission spectrum (+)	0.70	36.04****	0.0003	0.77
	Sampling effort (+)	0.07	4.26		
Neochromis spp. number	Water transparency (+)	0.79	89.69****	<0.0001	0.95
	Shore slope (−)	0.09	29.36***		
	Variation in shore slope (+)	0.04	6.33*		
	Sampling effort (+)	0.03	5.70*		
Nyererei spp. number§	Width of transmission spectrum (+)	0.60	19.14**	0.0011	0.60
Paralabidochromis spp. number	$T_{580\ nm}/T_{400\ nm}$‡ (−)	0.50	34.66***	0.0002	0.83
	Sampling effort (+)	0.28	23.24***		
	Nile perch abundance (−)	0.05	4.27		
Shannon-Wiener index of species diversity‖	− (No variable with $F > 4$)	−	−	−	−

*VBL, *Neochromis* "velvet black"/"blue scraper". †Maximum depth is the depth of the deepest point within 100 m around each station and a measure of the time for which the area around each station is flooded again after the desiccation of the lake. ‡$T_{400\ nm}$, transmission at 400 nm; $T_{580\ nm}$, transmission at 580 nm. §Nyererei complex is an undescribed genus (*7*, *33*). ‖See (*27*).

mission were much shallower (*26*). Finally, a similar relation occurs across water depths at one site between the number of syntopically breeding species (*y*, as measured by underwater mapping of spawning sites at the island with maximum transparency) and spectral width [$1/y = 0.23 + (-0.0003x)$, $F = 20.54$, $r^2 = 0.91$, $P = 0.045$]. In all three instances, color morph and species numbers increased with the breadth of the light spectrum. Under monochromatic light conditions in very turbid waters, only one drab-colored species was found in each of three genera that were species rich and colorful at places with broad light spectra [*Neochromis* (shallow-dwelling algivores), *Nyererei* complex (deeper-dwelling planktivores), *Paralabidochromis* (shallow- and deep-dwelling insectivores)], and only one color morph in a species that was polymorphic under broad spectra (*Neochromis* "velvet black"). At the same time, species number was not correlated with overall population density (Pearson's product moment correlation coefficient = 0.40, $P = 0.18$).

The effect of light conditions on mate choice based on coloration is the most likely explanation for our results. Increasing water turbidity reduces the diversity of color signals on both sides of the color spectrum. The reduced effectiveness of signals causes relaxation of sexual selection for color, with consequent loss of male nuptial coloration and erosion of species diversity due to breakdown of reproductive barriers. Effects usually associated with eutrophication that are detrimental to fish diversity, such as changes in resource abundance and diversity or reduction in oxygen concentrations, cannot explain the highly correlated patterns of diversity in trophically different genera of cichlid fish. Nor can they explain why niche width and interspecific niche overlap are larger at islands with fewer species (*27*). The sexual selection explanation is consistent with these and other field observations: We found a negative correlation between spectral bandwidth and the proportion of phenotypes that are intermediate in coloration between the red and the blue species in a widely distributed pair of sympatric sibling species (Pearson's correlation coefficient = −0.85, $n = 12$ islands, $P = 0.0005$). Whereas intermediate types were absent from all six places with bandwidth above 320 nm, 3 to 50% of the individuals were intermediate at the three sites where bandwidth was between 310 and 190 nm, and the majority (68 to 88%) of the individuals were intermediate at the three sites where bandwidth was less than 190 nm, suggesting amalgamation of gene pools under monochromatic light conditions at places with turbid water (*28*).

The effect of light conditions on coloration by way of sexual selection had been demonstrated before for other fish (*17*, *29*) and it had been shown that populations are more diverse when sexual selection is stronger (*23*). Here, we have linked these effects to patterns of species diversity. Speciation by sexual selection can explain the large number of cichlid species (in different colors) of each ecological and anatomical type in Lake Victoria (*2*, *3*). Because other traits that might function as mating barriers evolved less rapidly, coexistence of several hundred species relies on visual mate choice. Where eutrophication turns the lights off, ecological and species diversity erode rapidly.

As a consequence of deforestation and agricultural practices, water transparency in Lake Victoria has probably been decreasing since the 1920s (*10*), and loss of diversity possibly occurred already before the intensive investigations into the lake's fauna. Predation of Nile perch on primary consumers and detritus feeders now adds to the eutrophication rate (*30*), and the industrialization and urbanization that has followed the Nile perch fishing boom is causing further deterioration of water quality. Pollution also threatens the other African Great Lakes and, if not counteracted by management policies, will indeed destroy large parts of the unique example of vertebrate evolution that the lacustrine cichlids represent.

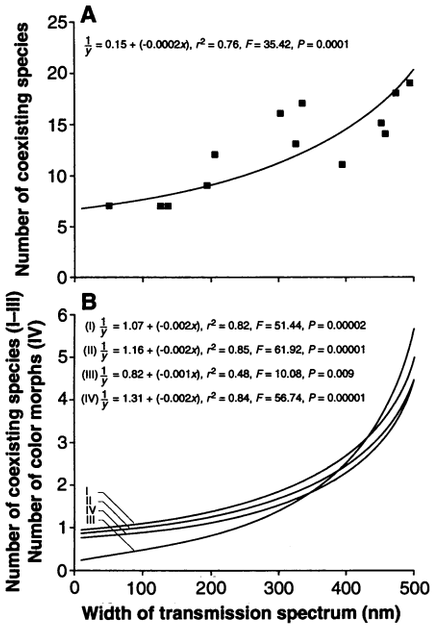

Fig. 2. Relation between bandwidth of the transmission spectrum and cichlid diversity. (**A**) Spectral bandwidth at 2 m water depth (*31*) and the number of coexisting haplochromine cichlid species. Each point represents one of 13 rocky islands along a south-north transect. (**B**) The same relation for the genera *Neochromis* (I), *Nyererei* complex (II), *Paralabidochromis* (III), and for the number of color morphs of *Neochromis* "velvet black"/"blue scraper" (IV).

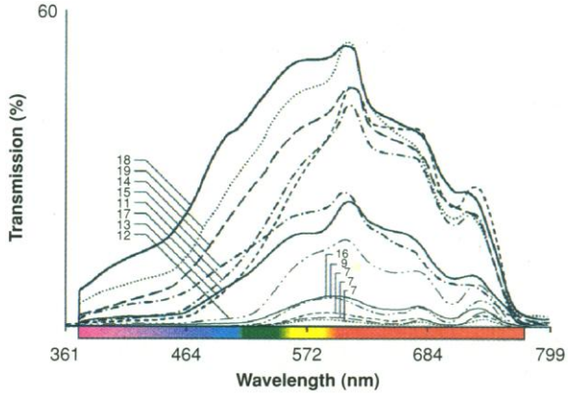

Fig. 3. Transmission spectra at a water depth of 2 m at the 13 research stations. Numerals indicate the number of coexisting haplochromine species for each station. Each curve is based on the mean from 10 spectral scans measured in 1996. The width of spectra at 10% transmission (nanometers) is related to turbidity (centimeter Secchi disc) as $y = 59.73 + 1.91x$, $r^2 = 0.88$, $F = 77.1$, $P < 0.00001$.

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March 1986 [M. De Beer, *Ann. Mus. R. Afr. Cent. Sci. Zool.* **257**, 57 (1989)] to 1.2 and 1.6 m in February to March 1995 and February to March 1996 (O. Seehausen, J. J. M. van Alphen, F. Witte, unpublished data).
12. In an experiment that was started in 1994, we crossed two females of *Platytaeniodus degeni* with one male *H. nyererei*. A phylogenetic analysis confirmed that they belong to two different genera (E. Lippitsch, O. Seehausen, N. Bouton, unpublished data). The F_1's were fully viable and did not suffer decreased fertility: nine females of the two F_1's produced clutch sizes of 2.64 ± 1.77 (n = 5) and 4.84 ± 2.57 (n = 4) fry per gram of body weight, versus 1.14 ± 1.29 (n = 10) in the *P. degeni* F_1 and 3.47 ± 2.43 (n = 7) in the *H. nyererei* F_1. The hybrid F_2 was also fully viable and fertile. Two females produced clutch sizes of 3.44 ± 3.34 fry per gram of body weight, and the first F_3 nest is now about to reach maturity. Sex ratios were 2:1 female biased in F_1 and F_2 hybrids as well as in both parental species. M. D. Crapon de Caprona and B. Fritzsch [*Neth. J. Zool.* **34**, 503 (1984)] found no difference in fertility between *Astatotilapia nubila* × *Haplochromis* "black lividus" hybrids and the parent species. Three further laboratory hybridizations that we did and three done elsewhere [M. D. Crapon de Caprona, *Ann. Mus. R. Afr. Cent. Sci. Zool.* **251**, 117 (1996)] yielded viable and, where tested (two intergeneric crosses in our lab), fertile offspring. Successful hybridization between genera of haplochromine cichlids, resulting in viable offspring, has also been reported from Lake Malawi [J. R. Stauffer, N. J. Bowers, T. D. Kocher, K. R. McKaye, *Copeia* (1996), p. 203].
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19. In one color morph, both sexes predominantly courted and responded to mates of their own morph (paired two-tailed t test: P = 0.00005 for females, n = 12, t = −5.03; P = 0.003 for males, n = 5, t = −4.31). In the other morph, females were unselective (P = 0.24, n = 12, t = 1.21) but males again courted predominantly females of their own morph (P = 0.021, n = 4, t = 2.85). In *N.* "blue scraper" both sexes are polymorphic. The morphs do not differ in ecology, nor in anatomy [O. Seehausen and N. Bouton, *Ecol. Freshw. Fish* **6**, 161 (1997)].
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25. We compared the male coloration of the *H. nyererei* population at Nyegezi rocks (Mwanza Gulf) in 1986 with that of the same population in 1993 through 1995. The red/blue ratio [610 nm/515 nm (*31*)] was significantly higher in the earlier year (1.18, SE = 0.14, n = 6 versus 0.75, SE = 0.17, n = 11; Z = −1.96, P = 0.05). Two males photographed in 1978 had an even higher red/blue ratio than those from 1986. Between 1986 and 1995 the water transparency at Nyegezi rocks had dropped from 3.2 to 1.2 m (*11*).
26. Minimum adequate regression model (and sign of correlation) for species number: area size (+) r^2 = 0.62, F = 19.48, spectral width (+) r^2 = 0.19, F = 6.72; model r^2 = 0.81, P = 0.007; model for color morph number: water transparency (+) r^2 = 0.69, F = 28.68, slope (−) r^2 = 0.13, F = 6.45; model r^2 = 0.82, P = 0.002.
27. The Shannon-Wiener index of species diversity is calculated from the number of species and the equitability of allotment of individuals among the species [C. J. Krebs, *Ecology, the Experimental Analysis of Distribution and Abundance* (Harper & Row, New York, 1972)]. The absence of a correlation with light conditions is explained by an inverse relation between species number and abundance equitability, indicating that resources are more equally alloted among species where species number is lower. This is supported by results of a study on resource utilization, which showed that niche width and interspecific niche overlap are larger were species number is lower (N. Bouton, O. Seehausen, J. J. M. van Alphen, *Ecol. Freshw. Fish*, in press).
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Structure and Function of a Squalene Cyclase

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The crystal structure of squalene-hopene cyclase from *Alicyclobacillus acidocaldarius* was determined at 2.9 angstrom resolution. The mechanism and sequence of this cyclase are closely related to those of 2,3-oxidosqualene cyclases that catalyze the cyclization step in cholesterol biosynthesis. The structure reveals a membrane protein with membrane-binding characteristics similar to those of prostaglandin-H_2 synthase, the only other reported protein of this type. The active site of the enzyme is located in a large central cavity that is of suitable size to bind squalene in its required conformation and that is lined by aromatic residues. The structure supports a mechanism in which the acid starting the reaction by protonating a carbon-carbon double bond is an aspartate that is coupled to a histidine. Numerous surface α helices are connected by characteristic QW-motifs (Q is glutamine and W is tryptophan) that tighten the protein structure, possibly for absorbing the reaction energy without structural damage.

The cyclization reactions catalyzed by squalene cyclases (S-cyclases) and 2,3-oxidosqualene cyclases (OS-cyclases) are highly complex (*1*) and give rise to various fused-ring compounds (*2*, *3*). Although these enzymes have been well studied earlier (*2*, *4*), present-day recombinant techniques have contributed much to the understanding of their function and reactivity (*1*, *5*). Early suggestions for the reaction mechanism favored a concerted process (*2*), whereas current hypotheses (*6*, *7*) dissect a series of carbocationic intermediates (Fig. 1). Recently, the OS-cyclases became targets for the development of antifungal and anticholesteremic drugs (*8*). The integral

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