

Color Polymorphism and Predation in a Lake Victoria Cichlid Fish

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Haplochromine cichlid fish have radiated into hundreds of species in East-African lakes, possibly driven by divergent sexual selection on body coloration. We studied the color polymorphic Lake Victoria cichlid *Neochromis omnicaeruleus*, in which a presumably ancestral phenotype with blue males and brown females co-occurs with two distinct classes of blotched phenotypes in both sexes. Similar blotch polymorphisms occur in other haplochromine species, and in all studied cases blotched females are much more common than blotched males. In *N. omnicaeruleus*, the near absence of blotched males seems to be partly due to genetic linkage to a dominant female determiner that turns blotched males into females. However, laboratory breeding suggests that blotched males should be much more common than observed. Here we studied whether differential predation on blotched males contributes to their scarcity. First, in a predation experiment with wild birds, blotched fish indeed incurred more predator attacks. Second, underwater observations revealed behavioral differences between the sexes, consistent with an additional predation risk for males. These data suggest that differential predation with regard to color pattern and sex may be an important selective force in the evolution and maintenance of this color polymorphism. However, we also carried out a population census which revealed that blotched males were rare already as juveniles. To explain the scarcity of blotched males in nature, we therefore have to invoke either selection against blotched males early in life, or a more complex genetic model. These results emphasize the need for further research on the ecology and genetics of this widespread color polymorphism in cichlid fish.

CICHLID fish are among the most species-rich of vertebrate clades. Sexual selection for color patterns has been invoked to explain the particularly rapid divergence of the haplochromines, a species-rich lineage of colorful cichlids that inhabit East-African lakes and rivers (Turner et al., 2001). Since intraspecific color variation resembles the differences observed between reproductively isolated species, it is thought that the latter evolve from the former (Seehausen et al., 1999a), likely driven by sexual selection for color (Seehausen and van Alphen, 1998; Couldridge and Alexander, 2002; Kidd et al., 2006).

We studied the so-called 'blotch' polymorphism, which occurs in several species of East-African cichlids (Seehausen, 1996; Konings, 2001) and differs from many other color polymorphisms in that it shows a strongly skewed distribution between the sexes (Lande et al., 2001). In the Lake Victoria haplochromine *Neochromis omnicaeruleus*, two classes of blotched morphs (white-blotched and orange-blotched) co-occur syntopically with a presumably ancestral 'plain' morph (Seehausen et al., 1999b). Whereas about half of all females are blotched, the vast majority of males have the plain phenotype (Seehausen et al., 1999b). This asymmetry may be partially explained by genetic linkage with dominant female determining sex genes (Seehausen et al., 1999b). However, given the crossing results of Seehausen

et al. (1999b; Table 1), and the high frequencies of blotched females in the field, blotched males should be considerably more common than observed. Moreover, blotched males are easily obtained in the laboratory by breeding from the rare wild blotched males and are fully viable and fertile (Seehausen et al., 1999b). Therefore, their scarcity in nature cannot be explained by the genetics of sex and color alone.

To the human observer, the blotched phenotypes are much more conspicuous than the plain phenotype (Fig. 1). Seehausen et al. (1999b) therefore hypothesized that differential predation on blotched males may contribute to their scarcity in nature. It is well established that predation can play a major role in the evolution and maintenance of color polymorphisms. For example, predator search images may enhance the survival of rare phenotypes (Clarke, 1962; Olendorf et al., 2006), habitat heterogeneity can drive the evolution of different camouflage patterns (Kettlewell, 1955; Forsman and Shine, 1995), spatial variation in predator communities affects sexually selected color traits (Endler, 1983), and aposematic species adopt different coloration patterns to join mimetic species assemblages in different parts of their range (Mallet, 1993). These and other mechanisms can maintain stable polymorphisms for many generations (Cain and Sheppard, 1954). However, polymorphisms may collapse when one morph has higher fitness

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Table 1. Phenotype Frequencies from *Neochromis omnicaruleus* Crosses. Expected phenotype frequencies of the offspring from crosses between different parental *N. omnicaruleus* phenotypes, predicted from the minimal adequate genetic model described by Seehausen et al. (1999b). First row and first column give the parental phenotypes and the presumed corresponding genotypes. P: plain; B: blotched. Allele *A* represents the blotch-linked dominant female determiner at either OB or WB locus. *M* and *m* are autosomal, color morph specific alleles that modify both color and sex. Boldscript indicates crosses that were carried out by Seehausen et al. (1999b).

Father		P aammxy		P aaMmxy		P aaMMxy		B (c) AaMMxy	
Mother P aammxx	♀	P	50	P	50	P	50	P	2.5
		B (a)	0	B (a)	0	B (a)	0	B (a)	0
		B (b)	0	B (b)	0	B (b)	0	B (b)	47.5
		B (c)	0	B (c)	0	B (c)	0	B (c)	0
		B (d)	0	B (d)	0	B (d)	0	B (d)	0
B (a) Aammxx	♀	P	25	P	25	P	25	P	12.5
		B (a)	50	B (a)	25	B (a)	0	B (a)	0
		B (b)	0	B (b)	24.35	B (b)	50	B (b)	48.70
		B (c)	0	B (c)	0	B (c)	0	B (c)	0
		B (d)	0	B (d)	0	B (d)	0	B (d)	25
B (b) AaMmxx	♀	P	25	P	25	P	25	P	2.6
		B (a)	25	B (a)	12.5	B (a)	0	B (a)	0
		B (b)	24.35	B (b)	24.35	B (b)	24.35	B (b)	23.08
		B (c)	0	B (c)	6.25	B (c)	12.5	B (c)	11.85
		B (d)	0	B (d)	0	B (d)	0	B (d)	23.70
B (c) AaMMxx	♀	P	25	P	25	P	25	P	2.6
		B (a)	0	B (a)	0	B (a)	0	B (a)	0
		B (b)	48.7	B (b)	24.35	B (b)	0	B (b)	0
		B (c)	0	B (c)	12.5	B (c)	25	B (c)	23.7
		B (d)	0	B (d)	0	B (d)	0	B (d)	23.7
B (d) AaMMxx	♀	P	25	P	25	P	25	P	23.7
		B (a)	0	B (a)	0	B (a)	0	B (a)	0
		B (b)	1.3	B (b)	0.65	B (b)	0	B (b)	0
		B (c)	0	B (c)	12.5	B (c)	25	B (c)	23.7
		B (d)	0	B (d)	0	B (d)	0	B (d)	2.6
B (d) AaMMxx	♀	P	0	P	0	P	0	P	0
		B (a)	0	B (a)	0	B (a)	0	B (a)	0
		B (b)	98.7	B (b)	48.7	B (b)	0	B (b)	0
		B (c)	0	B (c)	25	B (c)	50	B (c)	2.5
		B (d)	0	B (d)	0	B (d)	0	B (d)	47.5
B (d) AaMMxx	♂	P	0	P	0	P	0	P	0
		B (a)	0	B (a)	0	B (a)	0	B (a)	0
		B (b)	1.3	B (b)	1.3	B (b)	0	B (b)	0
		B (c)	0	B (c)	25	B (c)	50	B (c)	47.5
		B (d)	0	B (d)	0	B (d)	0	B (d)	2.5

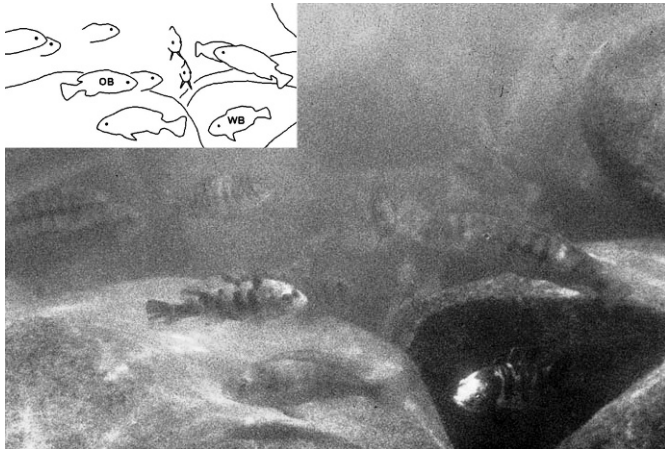


Fig. 1. *Neochromis omnicaeruleus* in its natural habitat at Makobe Island: the algae-covered rocks at ~1.5 meters depth. The two blotched individuals, one orange-blotched and one white-blotched, are better discernable than the nine plain fish.

than others and becomes fixed. Alternatively, strong assortative mating can turn color morphs into reproductively isolated species (Jiggins et al., 2001).

We investigated the hypothesis that differential predation contributes to the scarcity of blotched *N. omnicaeruleus* males in Lake Victoria, using three approaches. First, we exposed the three color morphs to avian predation in outdoor ponds. Second, we carried out underwater observations to determine whether behavioral differences between the sexes are consistent with an elevated predation risk for males. Third, we investigated the relative abundance of color morphs in both sexes over different age classes and water depths. If blotched fish suffer more from predation than plain morphs, we expect their numbers to decrease with age, and if males are affected more severely than females they should show the steepest decline. We recorded gonadal maturity stages of the fish as an indication of morph-specific life-history adaptations; high predation risk may select for earlier maturation (Van Noordwijk and De Jong, 1986). We investigated morph-specific depth distributions as a possible behavioral adaptation to predation risk. Visual hunters may preferentially forage in shallower water, whereas light conditions in deeper water make visual hunting difficult and may thus provide a safer environment for conspicuously colored fish. Finally, we compared the observed phenotype frequencies to those recorded by Seehausen et al. (1999b) between 1991 and 1996. As far as we know, these datasets yield the first long-term field study on phenotype frequencies in a cichlid color morph complex.

MATERIALS AND METHODS

Animals and study area.—All experiments and observations were carried out between November 2002 and April 2003. Field work was done at Makobe Island in the western Speke Gulf (Tanzania; Seehausen and Bouton, 1997), and fish from that same population were used in pond experiments. At Makobe the water is relatively clear (Secchi reading: mean \pm se = 223 \pm 9.5 cm; n = 43 measurements in the study period). In this population of *N. omnicaeruleus*, three distinct color morphs occur in both sexes. The putatively ancestral color morph, plain (P), has metallic blue or yellow males and brown or yellow females with dark vertical bars (Maan et al.,

2006a). Orange-blotched (OB) individuals have black blotches on an orange background when heterozygous, and become almost orange when homozygous. Black and white-blotched (WB) individuals have black blotches on a brassy to white background when heterozygous, and become almost black when homozygous.

Genetic model.—Based on laboratory crosses, Seehausen et al. (1999b) deduced a minimum genetic model to explain the different phenotypes (Table 1). Results indicate that WB and OB are tightly linked to two different x-linked dominant female determiners, and that the population also segregates for autosomal modifier genes with *M* and *m* alleles. *M* has an additive effect on the expression of the blotch color patterns and a recessive and epistatic effect on sex; when homozygous it negates the sex reversal effect of the x-linked female determiners (Seehausen et al., 1999b). The *M* alleles are color morph specific. As a result, at least eight different blotched phenotypes are theoretically possible in females and six in males, ranging from plain to either blotch homozygote, designated a, b, c, and d following Seehausen et al. (1999b). Given that the majority of blotched females in the lake are of the c type (heterozygous for the female determiner, homozygous for the modifier *M* allele), this model predicts that about the same proportion of all males should be blotched. This is because for every c female produced by a c mother, about one blotched male is produced (Table 1). Color morphs exhibit incomplete assortative mating preferences: whereas blotched males and both sexes of the plain morph show morph-assortative preferences, blotched females do not prefer blotched males (Seehausen et al., 1999b).

Predation experiment: experimental procedure.—The experiment was carried out at the Mwanza Station of the Tanzanian Fisheries Research Institute (TAFIRI), on the eastern shore of the Mwanza Gulf, Lake Victoria. Pied Kingfisher, *Ceryle rudis*, is abundant in this area; up to 30 individuals were present near our ponds at any one time. We took advantage of the presence of these birds for a color morph predation experiment using spontaneous attacks. Birds were not caught or handled in any way. Pied Kingfishers are natural predators of haplochromine cichlids (Douthwaite, 1976). We did not recognize the birds individually, but plumage differences between the sexes and between juveniles and adults confirmed that at least five different individuals were involved in successful predation events. We used an inflatable plastic pond of 110 x 110 cm inner surface. The bottom and sides were covered with an olive green plastic sheet, resembling the color of the algae cover on the rocks in the natural habitat of the fish. The pond was filled with water to 15 cm height. In pilot experiments we established that this water level was attractive to the birds as well as comfortable for the fish (fish swam over and underneath each other without apparent behavioral interference). The pond was placed in the shadow of a tree in which an observation hide-out was constructed two meters above ground. Around the pond we placed four perches on wooden poles of 1.5 m height to attract birds.

Fish were collected at 0.5–3 meters water depth at Makobe Island, using gillnets and hook and line, and subsequently kept in aquaria at the research station. Twelve fish, four of each color morph, were released in the pond the evening

before a trial. Groups were assembled such that the difference in mean size between color morph groups was minimal (standard length, measured to the nearest 0.1 mm; range 50.4–72.7 mm; this is within the size range of haplochromines eaten by Pied Kingfishers in nature; Douthwaite, 1976). Within experiments, the difference in mean size between the three morph groups did not exceed 1.6% (mean 0.7%).

Blotched males were too rare in nature to collect them in sufficient numbers for this experiment. We therefore used blotched females of the common OB(c) and WB(c) phenotypes, and plain blue males. Since territorial males may be more showy than females (see below), using blotched females and plain males was conservative with regard to testing the hypothesis that blotched color morphs suffer elevated avian predation pressure. We carried out six replicates of the test, using 72 different fish (24 of each color morph).

We kept the pond covered by mosquito mesh until observations started. Observations were carried out throughout the day between 0700 and 1800 hours, interrupted occasionally by inclement weather (rain, wind) and disturbances (grazing cattle). A trial was completed when six successful attacks had been recorded. This took two or three days per trial (average 15 hours and 22 minutes of observation; range: 9 hours and 27 minutes to 29 hours and 20 minutes). The proportion of successful attacks was 60%, which is similar to the behavior of kingfishers in natural (52%; Douthwaite, 1976) as well as experimental situations (70%; Labinger et al., 1991). Whenever the pond was not being observed, it was covered with mosquito mesh.

Predation experiment: activity observation.—We measured behavioral activity of fish in ten-minute observation periods spread evenly over the total observation period for each replicate. As a measure of activity we chose the number of times that a fish disturbed the water surface, because this could be unequivocally determined from the hide-out and attracted the attention of birds (Randle and Chapman, 2004). We collected between 60 and 180 minutes of behavioral observations per replicate experiment (mean \pm SE = 102 \pm 18 minutes). We could not recognize the fish individually and therefore recorded behavior by color morph. Behavioral differences between morphs could potentially be overshadowed by sexual differences. The experimental design addressed that problem in two ways. First, as explained above, we expected males to be more active than females. By using plain males and blotched females we avoided confounding the effects of behavior and color pattern. Second, we assumed that behavioral differences between the sexes would be associated with territoriality and reproduction. To assure males did not establish territories, shelters were not provided in the ponds, fish stayed in the ponds for only a few days, and we used males of slightly smaller sizes than would be reproductively active in nature.

Behavioral observation in nature.—To explain why blotched males are rare in nature, whereas blotched females are not, we hypothesized that territorial males are at an additional risk due to higher behavioral activity and/or more conspicuous behavior. We carried out focal observations of 26 adult males and nine adult females between 1.5 and 4 meters water depth at Makobe Island, using SCUBA. All males were territorial; they were observed for 15 minutes each. Females were observed for only ten minutes each because they moved

longer distances than males did, and were difficult to follow for longer periods of time. All observations were done between 0900 and 1300 h. We recorded frequencies of the following behaviors: swim, feed, chase, aggressive display (lateral and frontal display), and courtship display (quiver and leadswim in males; quiver, approach, and follow in females).

Estimating morph frequencies in nature.—We collected 490 individuals (274 males and 216 females) using gillnets with stretched mesh sizes of 10.5, 12.5, 16.5, and 18.5 mm. We set all nets at six depth ranges: 1.5, 2, 3, 4, 5, and 6 meters. Total sampling effort was 770 minutes in 12 days. Fish were photographed immediately after capture and put on ice. Females were subsequently measured ($n = 209$, standard length, to the nearest 0.1 mm) and dissected to determine gonadal maturation on a scale from 1 to 5 ($n = 204$, score 1: ovaries contain eggs of less than 0.5 mm diameter, score 5: eggs are ready to be laid). Female gonads mature gradually with age, with size at 50% maturity = 63 mm standard length (Seehausen et al., 1998). Once spawning has occurred, it takes typically between three and eight weeks to gradually mature a new batch of eggs, with no distinct spawning seasonality (Seehausen et al., 1998). The color morph abundances in both sexes were compared to data of four earlier study periods in the same population (1991: 478 individuals, 1992–1993: 560, 1995: 827, and 1996: 734; Seehausen et al., 1999b).

Data analysis.—To test whether kingfishers selected particular color morphs in the experiment, we compared the observed predation frequencies to expected frequencies using χ^2 -tests (SPSS 10.0, SPSS Inc.). We used two approaches. First, expected predation frequencies were assumed to be equal for all color morphs and to remain constant throughout a trial, disregarding the change in morph abundances after each successful bird attack. Second, expected predation frequencies were adjusted after each predation event, using the number of remaining fish of each color morph to generate the expected predation frequency. Pairwise comparison of predation rate between morphs was done with Wilcoxon signed ranks tests. Differences in behavioral activity were tested with a Friedman test; pairwise comparisons between morphs were done with Wilcoxon signed ranks tests. Male and female behavior in the field was translated into bouts per minute and compared using Mann–Whitney U tests.

The abundances of color morphs in different size and water depth classes, the interaction between size and depth, the relationship between size and gonadal maturation stage, and the effect of color morph on these relationships were analyzed with generalized linear models (GLM), using a Poisson distribution in R software (Ihaka and Gentleman, 1996; <http://www.r-project.org>). Significance was determined by F -tests examining the change in deviance following removal of variables. Test statistics were adjusted for under-dispersion (Venables and Ripley, 2002). Differences between color morphs in the distribution of individuals over gonadal stage classes were analyzed in an ordinal response model in R (Lindsey, 1995:59–61).

RESULTS

Selective bird predation on color morphs.—In each of the six trials, the blotched fish were preyed upon more than the

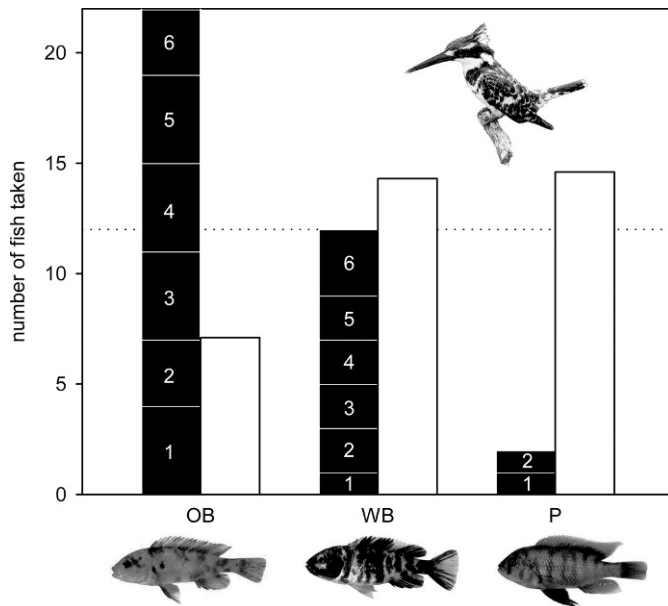


Fig. 2. Predation frequencies for orange-blotched (OB), white-blotched (WB), and plain (P) *N. omnicaeruleus* in the experiment. For each morph, black bars represent the total number of fish taken in each of the six trials; trial numbers are indicated. Open bars indicate the expected numbers of fish taken, adjusted for the changing morph proportions after each predation event. The dashed line indicates an even distribution.

plain morphs (Fig. 2). Out of 36 fish taken by kingfishers, 22 were orange blotched, 12 were white blotched, and only two were plain. These frequencies are significantly different from an even distribution ($\chi^2 = 16.67$, $df = 2$, $P < 0.001$) and from each other (Wilcoxon signed ranks test on six trials: OB vs. WB: $z = -2.06$, $P = 0.039$; WB vs. P: $z = -2.06$, $P = 0.039$; OB vs. P: $z = -2.23$, $P = 0.026$). We compared the observed predation frequencies to a frequency distribution under random predation, adjusted for the changing color morph abundances after each predation event. This yielded significant differences in all comparisons: three morphs together: $\chi^2 = 42.93$, $df = 2$, $P < 0.001$; OB and P: $\chi^2 = 26.19$, $df = 1$, $P < 0.001$; WB and P: $\chi^2 = 8.07$, $df = 1$, $P < 0.01$, OB and WB: $\chi^2 = 19.05$, $df = 1$, $P < 0.001$. There were no significant differences between trials in the numbers of fish of each morph that were predated ($\chi^2 \leq 4$, $df = 5$, $P > 0.50$). We did not detect differences in behavioral activity between the three color morphs during the experiments (Friedman test on medians per trial: $n = 6$, $df = 2$, $\chi^2 = 4.00$, $P = 0.135$). Pairwise comparison of morphs revealed a nonsignificant trend for white-blotched fish to be more active than plain fish (Wilcoxon signed ranks test; WB vs. P: $z = -1.783$, $P = 0.075$; WB vs. OB: $z = -1.572$, $P = 0.116$, OB vs. P: $z = -0.943$, $P = 0.345$). Territory settlement did not take place in the ponds, probably because shelters were absent, the time spent in the pond was short, and males were mostly juveniles (size at 50% maturity is 86 mm in males; Seehausen et al., 1998). Fish size did not affect predation probability: there was no difference in size between predated and non-predated fish (GLM, $F_{1,65} = 0.430$, $P = 0.514$).

Sexual dimorphism in behavior in the wild.—When observed in the wild, male and female *N. omnicaeruleus* did not differ significantly in the frequencies of swimming, feeding, or chasing behavior ($n_1 = 26$, $n_2 = 9$, Mann-Whitney U tests, swimming: $z = -1.17$, $P = 0.242$, feeding: $z = -1.37$, $P =$

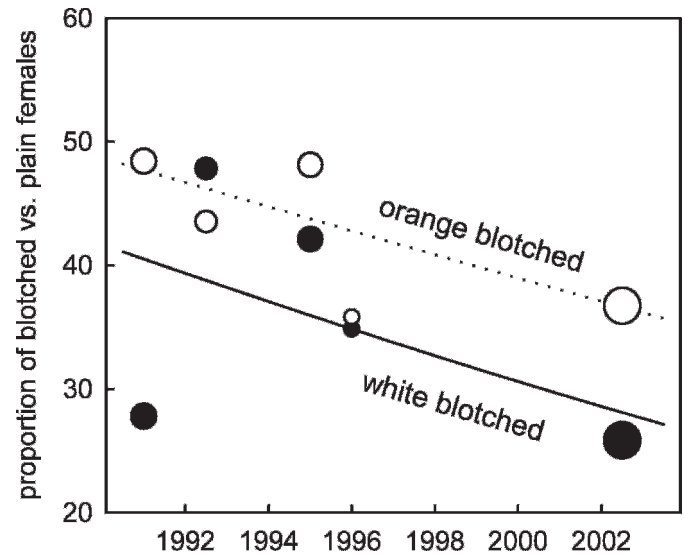


Fig. 3. The decrease in the proportion of *N. omnicaeruleus* blotched females between 1991 and 2003 at Makobe Island. Symbol sizes indicate sample sizes: 1991: 152 females, 1992–1993: 129, 1995: 146, 1996: 90, 2002–2003: 216.

0.172, chasing: $z = -0.61$, $P = 0.544$). Aggressive display and courtship behaviors, however, were more frequently observed in males than in females ($z = -2.65$, $P = 0.008$; $z = -2.55$, $P = 0.011$; $z = -2.227$, $P = 0.023$). None of the females observed engaged in courtship behavior.

Phenotype frequencies in the lake.—We caught 274 males (272 plain, one orange-blotched, and one white-blotched) and 216 females (112 plain, 65 orange-blotched, and 39 white-blotched). The proportion of blotched males (0.7%) was not significantly different from that measured by Seehausen et al. (1999b) between 1991 and 1996 (1.2%; $n_{91-96} = 2082$, $n_{02-03} = 274$, $\chi^2 = 0.429$, $P = 0.512$). The proportion of blotched females, however, had decreased significantly: 48.1% in 2002/2003 compared to 59.1% in 1990–1996 ($n_{91-96} = 516$, $n_{02-03} = 216$, $\chi^2 = 10.74$, $P = 0.001$). The decrease was significant for white-blotched females, from 25.6% in 1990–1996 to 18.1% in the present study ($\chi^2 = 6.43$, $df = 2$, $P = 0.040$), but not for orange-blotched females (from 33.5% to 30.1%; $\chi^2 = 1.14$, $df = 2$, $P = 0.565$). A combined GLM analysis, using the data of the four study periods of Seehausen et al. (1999b) and the present study, also revealed a significant decrease in the proportion of blotched females over the years (estimate = -0.044 ± 0.017 , $F_{1,3} = 6.85$, $P = 0.009$, Fig. 3).

The frequency of the *M* and *m* alleles at the putative autosomal locus that affects color had not changed: 8.7% of blotched females carried at least one *m* allele, which is not significantly different from the 10.5% reported by Seehausen et al. (1999b; using only the sample from 1991, for which the different blotched phenotypes were scored: $n_{91} = 86$, $n_{02-03} = 104$, $\chi^2 = 0.538$, $df = 1$, $P = 0.463$).

Distribution of color morphs over depth ranges and size classes.—Our male sample did not allow statistical comparison of the morph frequencies between different size and depth classes (SL and depth of the two blotched males: OB: 70.9 mm at 6 m, WB: 88.0 mm at 4 m). In the female sample, the proportion of blotched fish was not significantly

different between size classes ($F_{1,4} = 0.233$, $P = 0.629$) but increased with water depth ($F_{1,4} = 4.08$, $P = 0.043$). Testing for the blotched morphs separately (relative to the numbers of plain females) revealed weak but consistent trends (OB: $F_{1,4} = 2.82$, $P = 0.093$; WB: $F_{1,4} = 2.60$, $P = 0.107$). There was no correlation between depth and size (Pearson's $r = -0.007$, $P = 0.925$) and morphs did not differ in this respect (GLM explaining standard length by the interaction of water depth and color morph: $F_{1,207} = 0.006$, $P = 0.940$).

Gonadal maturation.—We studied gonadal maturation as an indicator of life-history adaptation to differences between color morphs in mortality rate. For all morphs, gonadal maturation stage increased with female standard length ($F_{1,202} = 31.14$, $P < 0.001$). There was a significant color morph effect; blotched females had a lower gonadal maturity score at a given size than plain females ($n_1 = 95$, $n_2 = 109$, $F_{1,201} = 5.433$, $P = 0.021$). The difference between blotched and plain females was due to trends for both categories of blotched females (OB vs. plain: $n_1 = 60$, $n_2 = 109$, $F_{1,166} = 3.084$, $P = 0.048$; WB vs. plain: $n_1 = 35$, $n_2 = 109$, $F_{1,141} = 3.042$, $P = 0.083$). There was no difference between white-blotched and orange-blotched females ($F_{1,92} = 0.224$, $P = 0.637$). An ordinal response model analysis, testing for different transition probabilities from one stage to the next, showed that the difference between plain and blotched females was entirely due to a lack of blotched stage-5 females. Transition probabilities for stages 1 to 4 were not significantly different between blotched and plain females ($F < 0.48$, $P > 0.49$) but the transition probability to stage 5 was significantly lower for blotched females ($F_{1,16} = 6.38$, $P = 0.012$).

DISCUSSION

The blotch polymorphism in *N. omnicaruleus* is linked to female and male determining sex genes (Seehausen et al., 1999b), and mathematical modeling suggests that the segregation of such genes in a population can lead to speciation by selection on sex reversal and sexual selection (Lande et al., 2001). Yet, complete assortative mating has not evolved between the morphs of *N. omnicaruleus*, possibly because the very low numbers of blotched males hinder the spread and fixation of female mating preferences for these male types (Seehausen et al., 1999b). The identification of the cause of this scarcity of males of the novel bright colors would, therefore, be a significant step towards understanding the conditions under which sympatric speciation can or cannot occur. Here we tested the hypothesis that blotched males are rare because they incur more predation than other fish.

Conflicting evidence for differential predation.—In our predation experiment, blotched *N. omnicaruleus* were attacked significantly more often by kingfishers than plain blue ones. Individuals of the orange-blotched morph were taken most often, followed by individuals of the white-blotched morph. Because these differences did not correspond to variation in behavioral activity, we conclude that in our experiment blotched coloration was more conspicuous to avian predators than plain blue coloration, and as a result attracted more kingfisher attacks.

Our experimental paradigm modeled a situation where fish would be seen by avian predators from above against the large algae-covered boulders that the fish graze on. Although cormorants, otters, and piscivorous fish may

approach their prey from the side, they would have a similar view, spotting the fish against a background of greenish water and rocks (Fig. 1). Our experiments should therefore model some component of natural predation, at least in shallow water, reasonably well. Some have suggested that blotched coloration may reduce rather than increase conspicuousness through a camouflaging or disruptive effect (Greenwood, 1956; Snoeks, 1994), but the large, algae-covered rocks in the natural habitat of these fish constitute a rather homogeneous visual background. Yet, to quantify the predation disadvantage of blotched fish in nature, further experiments should incorporate the role of background color and pattern, as well as the visual properties of different predators (Endler, 1978; Marshall, 2000).

Our behavioral observations in the lake indicated that adult males and females do not differ in the frequencies of swimming, feeding, or chasing behavior, but males showed significantly higher frequencies of both aggressive and courtship display. The finding that none of the observed females engaged in courtship is not surprising, given that only a small minority of adult females is ready to spawn at any one time (based on our measurements of gonadal maturity, we estimate this proportion at about 5–10%). Together with the results from the predation experiment, the behavioral difference between males and females is consistent with the hypothesis that blotched males suffer higher predation risk than plain males and than plain and blotched females. In cichlid fish, we only know of indirect evidence that male-specific behaviors increase predation risk: predator presence affects male fighting behavior in the South American species *Nannacara anomala* (Brick, 1998). Direct evidence comes from a variety of other taxa (e.g., arthropods [Jormalainen et al., 1995; Forsman and Appelqvist, 1999; Van Gossum et al., 2004], birds [Post and Gotmark, 2006], and amphibians and reptiles [Sullivan and Kwiatkowski, 2007]).

The relative abundances of color morphs at different depth ranges and in different size classes showed that blotched males were rare already in the smallest size class that we could catch. This implies that selective mortality with regard to both color and sex would have to occur early during maturation. Whereas morph-specific predation on juveniles is conceivable, sex-specific predation in this stage appears unlikely. Elevated courtship and aggressive behaviors would selectively expose males, but these behaviors are associated with territoriality, and male *N. omnicaruleus* do not become territorial until they reach 95 mm SL (Makobe Island, MEM and OS, unpubl. data).

Elevated predation risk for blotched males would exert selection for mechanisms that avoid the production of blotched males. This could be achieved by female mating decisions; blotched females minimize the number of blotched sons by mating with plain males of the *mm* genotype (Table 1). The phenotype frequencies among blotched females in nature, however, do not support this. Matings between blotched females and plain *mm* males would result in much higher frequencies of blotched females of the a and b types (carrying at least one copy of the recessive allele at the M locus) and much lower frequencies of types c and d (homozygous for the dominant M allele), than we observed. In fact, the data rather suggest that blotched females frequently mate with the rare blotched males; blotched d females (homozygous at both loci),

although rare, are about nine times more abundant than expected if heterozygous blotched females (a, b, c) mated at random (pooled dataset, 732 females and 2356 males, Fisher's Exact test: $P = 0.01$). Moreover, in laboratory mate-choice experiments, blotched females did not show any mating preferences (Seehausen et al., 1999b). Thus, female mating decisions cannot explain the low frequencies of blotched males in nature. Another possible mechanism would be a suppressed expression of the blotched coloration in males, allowing males to carry and transmit the blotch and modifier genes without being exposed to increased predation. Laboratory crosses did not reveal evidence for this, but, as noted already (Seehausen et al., 1999b), the power to detect such effects was limited.

Whereas our results suggest that differential predation with regard to color and sex could exert selection towards lower numbers of blotched males in the lake, the mechanisms proposed above are inconsistent with some of the data presented here and elsewhere (Seehausen et al., 1999b). To resolve the inconsistencies, assessment of mating behavior and genotype frequencies in nature is required. Moreover, extensive pedigree analyses in the laboratory should determine whether the genetics underlying the blotch polymorphism are more complex than the minimum model described by Seehausen et al. (1999b).

Spatial and temporal fluctuations of phenotype frequencies.—

The proportion of blotched females significantly decreased over a period of 12 years. This may be associated with a decrease in water clarity. Secchi disk measurements suggest that transparency at Makobe Island has decreased between 1991 and 2003 (partial correlation controlling for seasonal fluctuations: Pearson's $r = -0.19$, $n = 96$, $P = 0.071$), which corresponds to the overall transparency decrease in the lake during the last decades (Witte et al., 1999). Such an association would be consistent with the general trend that blotched polymorphisms in haplochromines are confined to clear water environments (Seehausen and Bouton, 1996), and may eventually lead to the complete disappearance of blotched phenotypes from this and other populations. Together with our finding that blotched fish suffer a higher predation risk than plain conspecifics, their confinement to clear waters presents a paradox: the hunting success of visually orienting fish predators is generally positively correlated with water transparency (Cezilly, 1992; Johnson and Hines, 1999; but see Reyer et al., 1988), affecting conspicuously colored individuals disproportionately (Moyaho et al., 2004). Moreover, in southeastern Lake Victoria piscivorous birds tend to be more abundant near clear waters (Seehausen et al., 1997). We found a small but significant increase in the proportion of blotched females towards deeper water. At Makobe Island, the light intensity at 5 m depth is about 35% of the light intensity at 2 m depth (Maan et al., 2006b), where plain *N. omnicaeruleus* is most abundant. Therefore, the difference in conspicuousness between the plain and blotched color patterns may be reduced in deeper water. The effect of light environment on differential predation on blotched cichlid morphs requires further study. For example, water turbidity may increase background homogeneity, possibly facilitating predator detection of blotched phenotypes but not plain ones (Cain and Sheppard, 1954; Bond and Kamil, 2006).

The difference in gonadal development between blotched and plain females may indicate a difference in life history,

but the data are inconclusive. A relatively steep increase of gonadal maturity with size would indicate an earlier onset of reproduction, consistent with adaptation to a higher adult mortality rate (Van Noordwijk and De Jong, 1986). However, the deficit of blotched females in stage 5 of gonadal maturity can be explained in two ways: blotched females may either mature more slowly than plain females, or they may start brooding sooner after reaching stage 5. The data do not allow rejection of either of these alternatives.

Our data indicate that differential predation with regard to color pattern, and possibly sex, could be an important selective force in the evolution and maintenance of mating preferences and color variation. This is the first evidence to suggest that natural selection may hinder the establishment of conspicuous color morphs in haplochromine cichlid fish. However, whereas blotched phenotypes suffered elevated predation under experimental conditions, phenotype distributions in a natural population revealed no evidence for differential mortality between morphs. These results emphasize the need for further research on the ecology and genetics of this widespread color polymorphism in cichlid fish.

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