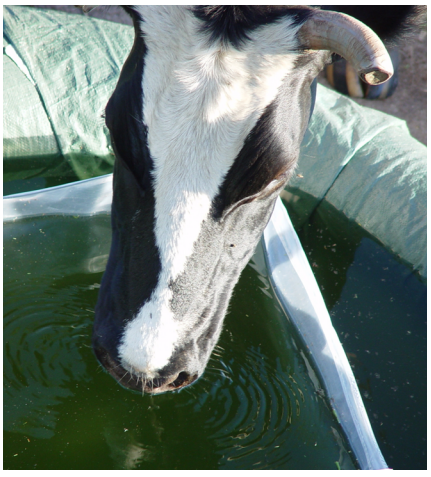
Sexual selection and speciation: mechanisms in Lake Victoria cichlid fish

Maan, M.E.

Chapter 8

'Obscure tints have often been developed through natural selection for the sake of protection, and the acquirement through sexual selection of conspicuous colors, appears to have been sometimes checked from the danger thus incurred.'

Darwin, 1871



Differential predation in a colour polymorphic Lake Victoria cichlid

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(submitted)

Haplochromine cichlid fish have radiated into hundreds of species in East-African lakes, possibly driven by divergent sexual selection on body coloration. We study the colour 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 is 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 study whether differential predation on blotched males contributes to their scarcity, using three approaches. First, we carried out a predation experiment with wild birds and found that blotched fish indeed incur more predator attacks. Second, we observed behavioural differences between the sexes in the lake, consistent with additional predation risk for males. Third, we carried out a population census which indicated that blotched males are rare already as juveniles. This means that to explain the scarcity of blotched males in nature we have to invoke either selection against blotched males early in life, or a more complex genetic model. Our data suggest that differential predation with regard to colour pattern and sex may be an important selective force in the evolution and maintenance of haplochromine colour polymorphisms.

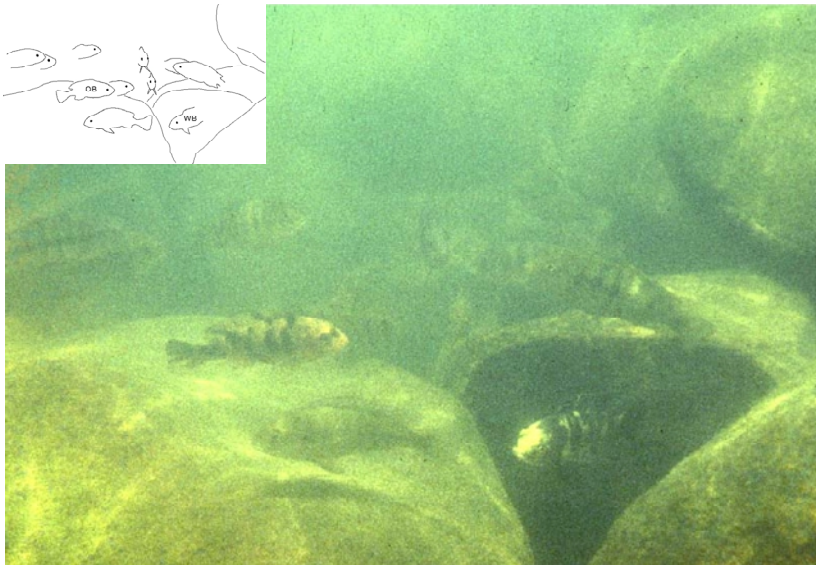

Figure 8.1
Neochromis omnicaeruleus in its natural habitat at Makobe Island: the algae-covered rocks at ~1.5 meters depth. The two blotched individuals, one orange-blotched and one white-blotched, are better discernable than the nine plain fish. Photo by OS.

Introduction

The cichlid fish of the Great Lakes of East Africa are textbook examples of explosive speciation. In particular, the haplochromines of Lakes Malawi and Victoria have radiated extremely fast (Meyer 1993; Johnson et al. 1996; Nagl et al. 2000; Won et al. 2005). The cichlid feeding apparatus and its genetic architecture facilitated ecological niche differentiation (Liem 1973; Galis & Drucker 1996; Albertson et al. 2003), reducing competitive exclusion and allowing species diversity to build up. Much of the large scale ecological differentiation that characterises these species flocks occurred in the early phases of radiation (Danley & Kocher 2001; Streelman & Danley 2003). Among closely related species however, ecological differentiation is subtle, but they usually differ strikingly in body coloration (Albertson et al. 1999; Seehausen et al. 1999b; Allender et al. 2003; Knight & Turner 2004). Sexual selection on body coloration has probably been important in the evolution of bright colour patterns (Seehausen et al. 1999c; Chapter 2; Pauers et al. 2004) and may have contributed to the rapid speciation of haplochromines (Dominey 1984).

Blotch polymorphisms are widespread among haplochromine cichlids (Seehausen 1996; Konings 2001). In the Lake Victoria haplochromine *Neochromis omnicaeruleus*, two classes of blotched morphs co-occur with a presumably ancestral 'plain' type: white-blotched and orange-blotched (Seehausen et al. 1999b). As in other haplochromines (Lande et al. 2001), the phenotype distribution is strongly skewed between the sexes: in our study population, about half of all females is blotched whereas the vast majority of males have the plain phenotype (Seehausen et al. 1999b). This asymmetry is due to genetic linkage with female determining sex genes (Seehausen et al. 1999b). However, based on the genetic model proposed by Seehausen et al. (1999b), 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 colour alone.

To the human observer, the blotched phenotypes are much more conspicuous than the plain ones (Figure 8.1). Seehausen et al. (1999b) therefore hypothesised that differential predation on blotched males may contribute to their scarcity in nature. Here, we investigate this hypothesis, using three approaches. First, under semi-natural conditions, we expose the three colour morphs to avian predation. Second, we carry out underwater observations to determine whether behavioural differences between the sexes are consistent with an elevated predation risk for males. Third, we investigate the relative abundance of colour morphs in both sexes over different age classes and water depths. If blotched fishes suffer more from predation than plain ones, we expect their numbers to decrease with age, and if males are affected more severely than females they should show the steepest decline. We record the gonadal maturity stages of the fish as an indication of morph-specific life-history adaptations: high predation risk may select for ear-

lier maturation (Van Noordwijk & De Jong 1986). We investigate morph-specific depth distributions as a possible behavioural adaptation to predation risk. Visual hunters may preferentially forage in shallower water, whereas light conditions in deeper water make visual hunting difficult and thus provide a safer environment for conspicuously coloured fish. Finally, we compare the observed phenotype frequencies to those recorded by Seehausen et al. (1999b) between 1991 and 1996. Together, these datasets yield one of the very few long-term field studies on phenotype frequencies in a cichlid morph complex.

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 & Bouton 1997) and fish from that same population were used in pond experiments. At Makobe the water is relatively clear (Secchi reading: mean±se=223±9.5 cm; n=43 measurements in the study period). In this population of *Neochromis omnicaeruleus*, three distinct colour morphs occur in both sexes (Figure 8.4 on page 128). The putatively ancestral colour morph, 'plain' (P), has metallic blue males and brown females with dark vertical bars. 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 minimal genetic model to explain the different phenotypes (see Appendix 8.1). 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 colour 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 colour morph specific. As a result, at least eight different blotched phenotypes are theoretically possible in females and six in males, designated a, b, c and d following Seehausen et al. (1999b). Given that the majority of blotched females in the lake is 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 as well (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 kingfishers, *Ceryle rudis*, were abundant in this area: up to 30 individuals were present near our ponds at any one time. We used spontaneous attacks in the experiment; birds were not caught or handled in any way. Pied kingfishers are natural predators of haplochromine cichlids (Douthwaite 1976). We did not recognise 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 110·110 cm inner surface, covered with a dull green plastic sheet, resembling the colour of the algae cover on the rocks in the natural habitat of the fish. The ponds were 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. The pond was placed in the shadow of a tree in which an observation hide-out was constructed 2 m above ground. Around the pond we placed 4 wooden poles of 1.5 m long to attract birds.

Fish were collected at 0.5 to 3 m 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 colour morph, were released in the ponds the evening before a trial. Groups were assembled such that the size differences between colour morph groups were 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 are far 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 behave more showy than females (see below), using blotched females and plain males was conservative with regard to testing the hypothesis that blotched colour morphs suffer higher avian predation pressure. We carried out six replicates of the test.

We kept the pond covered by mosquito mesh until observations started. Observations were carried out throughout the day between 07:00 and 18:00, interrupted occasionally by heavy weather conditions (rain, wind) and disturbances (grazing cattle). A trial was completed when 6 successful attacks had been observed. This took two or three days per trial (average 15 hours and 22 minutes of observation; range 9h27m-29h20m). The proportion of successful attacks was 60%, which is similar to the behaviour of pied kingfishers in natural (52%; Douthwaite 1976) as well in as experimental (70%; Labinger et al. 1991) situations. Whenever the pond was not being observed, it was covered.

Activity observation

We measured behavioural activity of the fish in 10-minute observation periods spread evenly over the total observation period for a replicate. As 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. We collected between 60 and 180 minutes of behavioural observation per replicate experiment (mean±se=102±18 minutes). We could not recognise the fish individually and therefore recorded behaviour by colour morph.

Behavioural observation in nature

To explain why blotched males, and not blotched females, are rare in nature, we hypothesised that territorial males are at an additional risk due to higher behavioural activity. Therefore, we carried out focal observations of 26 adult males and 9 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 10 minutes each because they moved around further than males did, and were impossible to follow for longer periods of time. All observations were done between 09:00 and 13:00 h. We recorded frequencies of the following behaviours: 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. 209 Females were subsequently measured (standard length, to the nearest 0.1 mm) and 204 of these were dissected to determine gonadal maturation on a scale from 1 to 5 (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 = 63mm standard length (Seehausen et al. 1998a). Once spawning has occurred, it takes typically between 3 and 8 weeks to gradually mature a new batch of eggs, with no distinct spawning seasonality (Seehausen et al. 1998a).

The colour morph abundances in both sexes were compared to four earlier samples taken in the same population: in 1991 (478 individuals), 1992-1993 (560), 1995 (827) and 1996 (734) (Seehausen et al. 1999b).

Data analysis

Observed predation frequencies in the experiment were compared to two different sets of expected frequencies (using Chi^2-tests in SPSS 10.0, SPSS Inc.). In the first set, expected predation frequencies were assumed to be equal for all colour morphs and to remain constant through each replicate experiment, independent

of changing morph abundances after each successful bird attack. In the second set, expected predation frequencies were adjusted after each predation event, using the number of remaining fish of each colour morph as the measure of expected predation frequency. The abundances of colour 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 colour morph on these relationships, were analysed with generalised linear models (GLM) using a Poisson distribution, in R software (Ihaka & 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 underdispersion (Venables & Ripley 2002). Differences between colour morphs in the distribution of individuals over gonadal stage classes were analysed in an ordinal response model in R (Lindsey 1995, pp. 59-61).

Results

Selective bird predation on colour morphs

In each of the six trials, the blotched fish were preyed upon more than the plain ones (Figure 8.2). Out of 36 fish taken by kingfishers, 22 were orange blotched, 12 were white blotched and only 2 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: WB vs. OB: Z=-2.06, p=0.039; WB

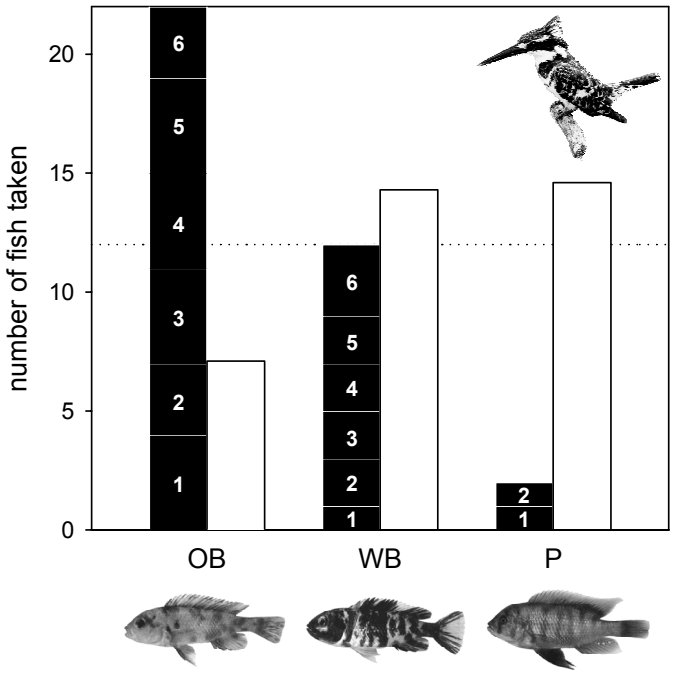

Figure 8.2 Predation frequencies for orange blotched (OB), white blotched (WB) and plain (P) *N. omnicaeruleus* in the experiment. For each morph, black bars represent the total number of fish taken in each of the six trials; trial numbers are indicated. Open bars indicate the expected numbers of fish taken, adjusted for the changing morph proportions after each predation event. The dashed line indicates an even distribution.

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 colour 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 behavioural activity between the three colour morphs during the experiments (Friedman test on medians per trial: n=6, df=2, Chi^2=4, p=0.14). Pairwise comparison of morphs revealed a trend for white-blotched fish to be more active than plain fish. (Wilcoxon signed ranks test: Z=-1.783, p=0.075 [WB vs. OB: Z=-1.572, p=0.12, OB vs. P: Z=-0.943, p=0.35]). 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. 1998a). Fish size did not affect predation probability: there was no difference in size between predated and non-predated fish (GLM, $F_{1,65}$=0.43, p=0.51).

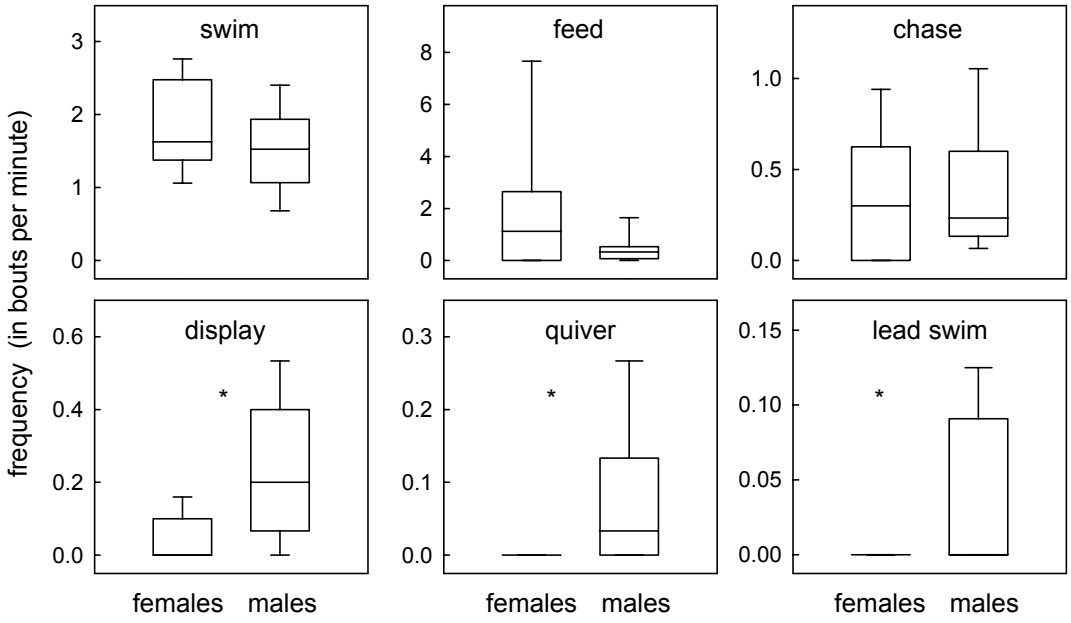

Figure 8.3 Behaviour of *N. omnicaeruleus* males (26) and females (9) at Makobe Island.

Sexual dimorphism in behaviour 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 behaviour (n_1=26, n_2=9, Mann-Whitney U tests, swimming: Z=-1.17, p=0.24, feeding: Z=-1.37, p=0.17, chasing: Z=-0.61, p=0.54; Figure 8.3, upper panels). Aggressive display and courtship behaviours 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; Figure 8.3, lower panels). Female courtship behaviour was not observed.

Distribution of colour morphs over depth ranges and size classes

We caught 274 males (272 plain, 1 orange-blotched and 1 white-blotched) and 216 females (112 plain, 65 orange-blotched and 39 white-blotched)(Figure 8.4). 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%; $n_{91\text{-}96}$=2082,

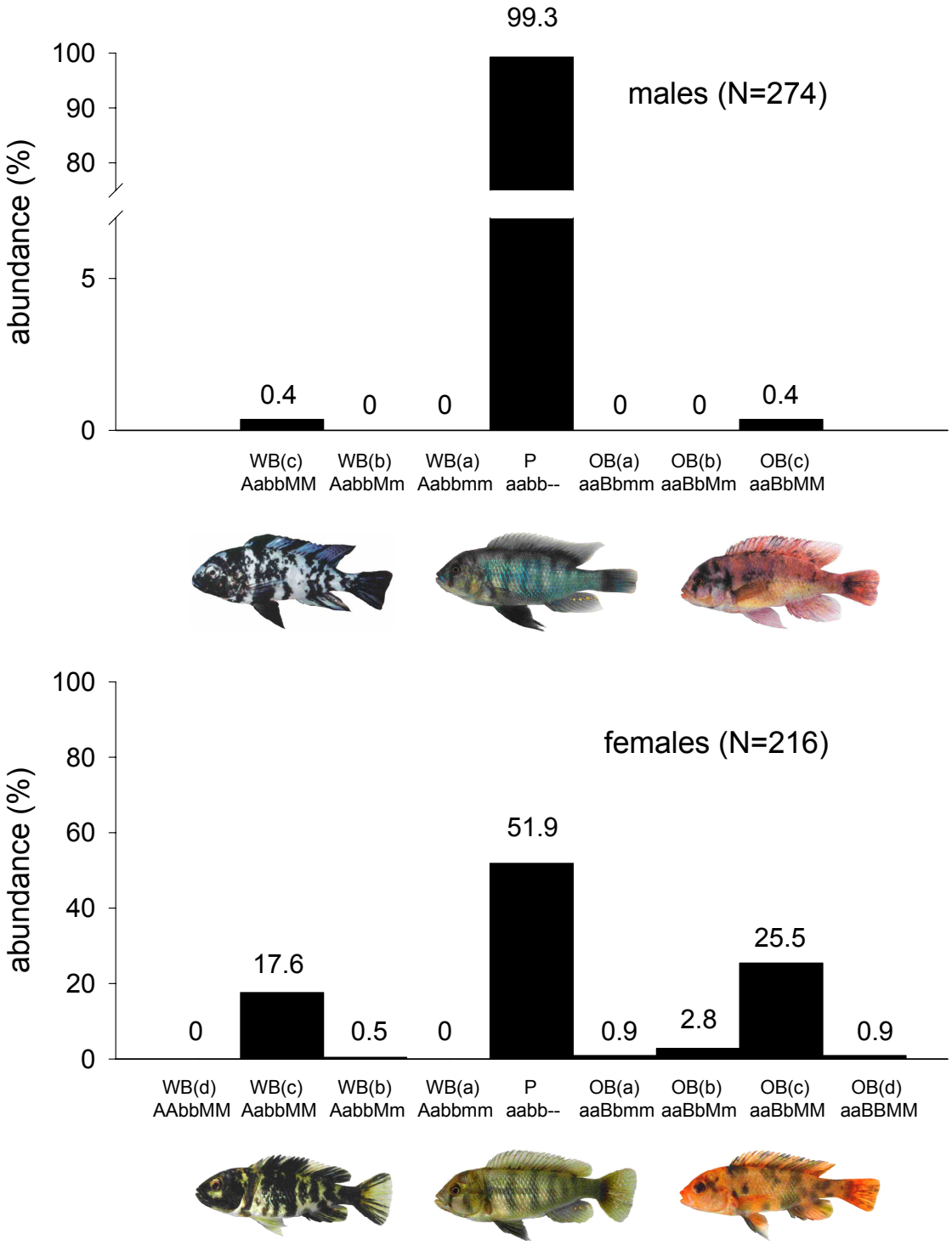

Figure 8.4. Abundances of *N. omnicaeruleus* phenotypes, with presumed genotypes, at Makobe Island. A,a: alleles at x-linked WB locus; B,b: alleles at x-linked OB locus; M,m: alleles at autosomal locus. For details on genotypes, see Methods text and Appendix 8.1.

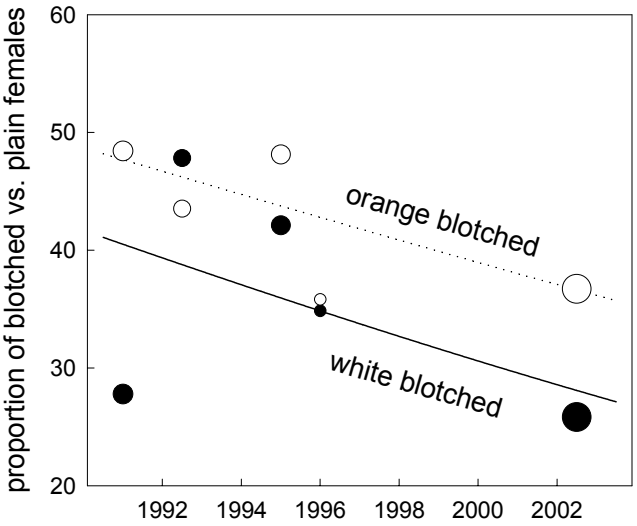

Figure 8.5. The decrease in the proportion of *N. omnicaeruleus* blotched females between 1991 and 2003 at Makobe Island. Symbol sizes indicate sample sizes: 1991:152 females, 1992-1993:129, 1995: 146, 1996: 90, 2002-2003: 216.

$n_{02\text{-}03}$=274, Chi^2=0.43, p=0.51). The proportion of blotched females however had decreased significantly: 48.1% in 2002/2003 compared to 59.1% in 1990-1996 ($n_{91\text{-}96}$=516, $n_{02\text{-}03}$=*216*, Chi^2=10.74, p=0.001, Figure 8.5). 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=0.04), but not for orange-blotched females (from 33.5% to 30.1%; Chi^2=1.14, df=2, p=0.56). A combined GLM analysis, using the four samples of Seehausen et al. (1999b) and the present study, showed 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, Figure 8.5).

The frequency of the *M* and *m* alleles at the putative autosomal locus that affects colour, 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_{1991}=86, $n_{2002\text{-}2003}$=104, Chi^2=0.55, df=1, p=0.36).

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 (Figure 8.6), the proportion of blotched fish was not significantly different between size classes ($F_{1,4}$=0.23, p=0.63) 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: $F_{1,4}$=2.6, p=0.11). There was no correlation between depth and size (Pearson's r=-0.01, p=0.93) and morphs did not differ in this respect (GLM explaining standard length by the interaction of water depth and colour morph: $F_{1,207}$<0.1, p=0.94).

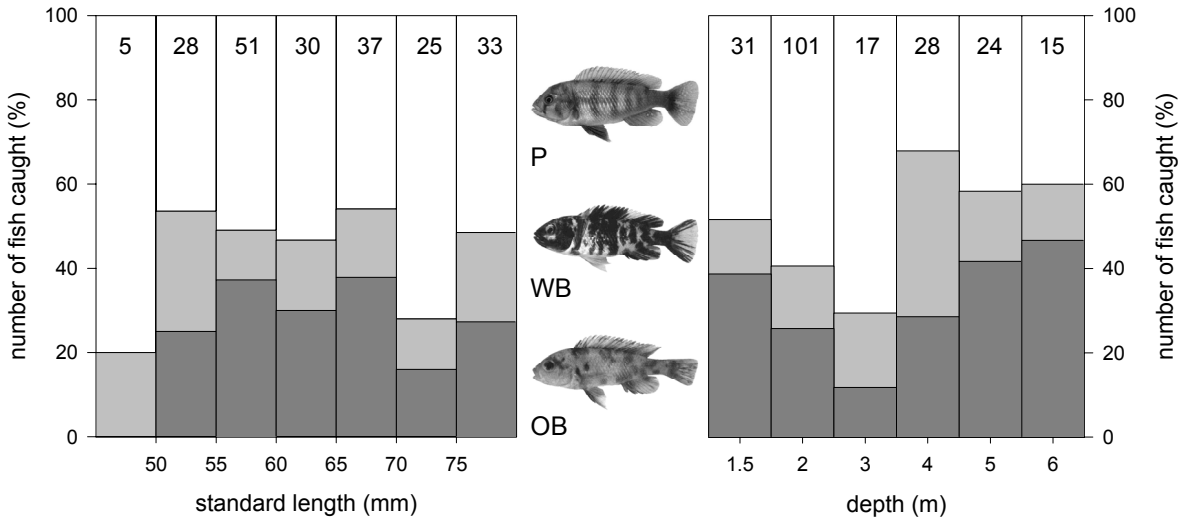

Figure 8.6. The numbers of females of the three *N. omnicaeruleus* colour morphs in different size (left panel) and depth (right panel) classes at Makobe Island. Numbers in the graphs indicate the sample size for each category.

Gonadal maturation

We studied gonadal maturation as an indicator for life-history adaptation to differences between colour 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 weak colour morph effect: blotched females had a lower gonadal maturity score at a given size than plain females: n_1=95, n_2=109, $F_{1,201}$=3.63, p=0.058. The difference between blotched and plain females was due to weak trends for both categories of blotched females (OB vs. plain: n_1=60, n_2=109, $F_{1,166}$=2.88, p=0.092; WB vs. plain: n_1=35, n_2=109, $F_{1,141}$=2.50, p=0.12). There was no difference between white-blotched and orange-blotched females ($F_{1,92}$=0.13, p=0.72). 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: blotched versus plain: $F_{1,16}$=6.38, p=0.012.

Discussion

Conflicting evidence for differential predation

In our predation experiment, blotched *Neochromis omnicaeruleus* were attacked significantly more often by pied 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 behavioural 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 modelled 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. 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 colour and pattern, as well as the visual properties of different predators (Marshall 2000).

Our behavioural observations in the lake indicated that adult males and females do not differ in the frequencies of swimming, feeding or chasing behaviour, but males showed significantly higher frequencies of both aggressive and courtship display. Together with the results from the predation experiment, this is consistent with the hypothesis that blotched males suffer higher predation risk than plain males and than plain and blotched females.

The relative abundances of colour morphs at different depth ranges and in different size classes however, 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 colour 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: although elevated courtship and aggressive behaviours would selectively expose males, these behaviours are associated with territoriality, and male *N. omnicaeruleus* do not become territorial until they reach 95 mm SL (Makobe Island, unpublished data).

Elevated predation risk for blotched males is likely to exert strong selection for mechanisms that avoid the production of blotched males. This could be achieved by female mating decisions: blotched females minimise the number of blotched sons by mating with plain males (Appendix 8.1). The phenotype frequencies among blotched females in nature however do not support this. Matings between blotched females and plain 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 9 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 there (Seehausen et al. 1999b), the power to detect such effects was limited.

Whereas our results suggest that differential predation with regard to colour and sex exerts 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). Progress requires assessment of mating behaviour and genotype frequencies in nature, as well as more extensive pedigree analyses in the laboratory: our results indicate that the genetics underlying the blotch polymorphism may be more complex than the minimal 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 twelve 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 & Bouton 1996). 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 & Hines 1999; but see Reyer et al. 1988), affecting conspicuously coloured individuals disproportionally (Moyaho et al. 2004). Moreover, we found a small but significant increase in the proportion of blotched females towards deeper water. At Makobe Island, the light intensity at 5 meters depth is ~25% of the light intensity at 2 meters depth (Chapter 5), where plain *N. omnicaeruleus* is most abundant. Therefore, the difference in conspicuousness between the plain and blotched colour patterns may be reduced in deeper water. These observations indicate that the effect of light environment on differential predation on blotched fish requires further study. For example, water turbidity may increase background homogeneity, possibly facilitating predator detection of blotched phenotypes but not plain ones. Total predator abundance is unlikely to explain the distribution of blotched fish within or between populations, because predator densities are highest in shallow and clear waters.

The persistence of the blotch polymorphism in the Makobe population requires a compensating advantage. Behavioural observations in the laboratory show that blotched females are more aggressive than plain ones (K. Pyle & O.

Seehausen, unpublished data) and that they dominate the latter in dyadic interactions (P.D. Dijkstra & T.G. G. Groothuis, unpublished data). This behavioural difference may be important for female reproductive success, especially during the mouthbrooding and fry-guarding phases of reproduction, when female aggressiveness is likely to enhance offspring survival. It may also give blotched females an advantage in competition for shelters with plain females. Together with the lower predation risk in deeper water, this may explain why the proportion of blotched females did not decrease with size. Finally, 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 & 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 reproducing sooner after reaching stage 5. The data do not allow rejection of either of these alternatives.

Conclusion

Whereas mathematical modelling has shown that the haplochromine blotch polymorphism can lead to speciation by sexual selection and sex ratio selection (Lande et al. 2001), complete assortative mating has not evolved between the morphs of *N. omnicaeruleus* (Seehausen et al. 1999b). Possibly, the very low numbers of blotched males hinder the spread and fixation of female mating preferences for these male types. Identification of the cause of the scarcity of blotched males would therefore be a significant step towards understanding the conditions under which reproductive isolation can or cannot evolve.

We found that blotched phenotypes suffer increased predation in experimental conditions, but that phenotype distributions in a natural population are inconsistent with differential mortality between morphs. Therefore, this study does not present a conclusive explanation for the dynamics of the *N. omnicaeruleus* blotch polymorphism. In fact, our results emphasise the need for further research of the underlying genetics. Our study also indicates that differential predation with regard to colour pattern and possibly sex, could be an important selective force in the evolution and maintenance of mating preferences and colour gene expression. As such, it presents the first evidence to indicate that natural selection may hinder the establishment of conspicuous colour morphs in haplochromine cichlid fish.

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Appendix 8.1. The expected phenotype frequencies of the offspring from crosses between different parental *N. omnicaeruleus* 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, colour morph specific alleles that modify both colour and sex. *M* affects WB or OB expression and compensates the sex reversal effect of the *A* allele. As a result, for both OB and WB, at least four different blotched phenotypes are theoretically possible in females and three in males, designated a, b, c and d following Seehausen et al. (1999b). For boxes printed in boldscript, the expected offspring frequencies were consistent with the outcome of laboratory crosses presented in Seehausen et al. (1999b). There are two exceptions to Mendelian inheritance, yielding numbers that deviate from multiples of 6.25%: i) incomplete dominance of the *M* allele: only 2.6% of the AaMMxy genotypes are rescued B(b) males, the rest are B(b) females; and ii) recombination between the x and y chromosomes, estimated to be about 5% (Seehausen et al. 1999b).

Father / Mother		P aammxy		P aaMmxy		P aaMMxy		B(c) AaMMxy	
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
	♂	P	50	P	50	P	50	P	47.5
		B (a)	0	B (a)	0	B (a)	0	B (a)	0
		B (b)	0	B (b)	0	B (b)	0	B (b)	2.5
		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
	♂	P	25	P	25	P	25	P	12.5
		B (a)	0	B (a)	0	B (a)	0	B (a)	0
		B (b)	0	B (b)	0.65	B (b)	0	B (b)	1.3
		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 (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
	♂	P	25	P	25	P	25	P	23.70
		B (a)	0	B (a)	0	B (a)	0	B (a)	0
		B (b)	0.65	B (b)	0.65	B (b)	0.65	B (b)	0.62
		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)	2.6
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
	♂	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
	♂	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