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Sexual selection and speciation: mechanisms in Lake Victoria cichlid fish

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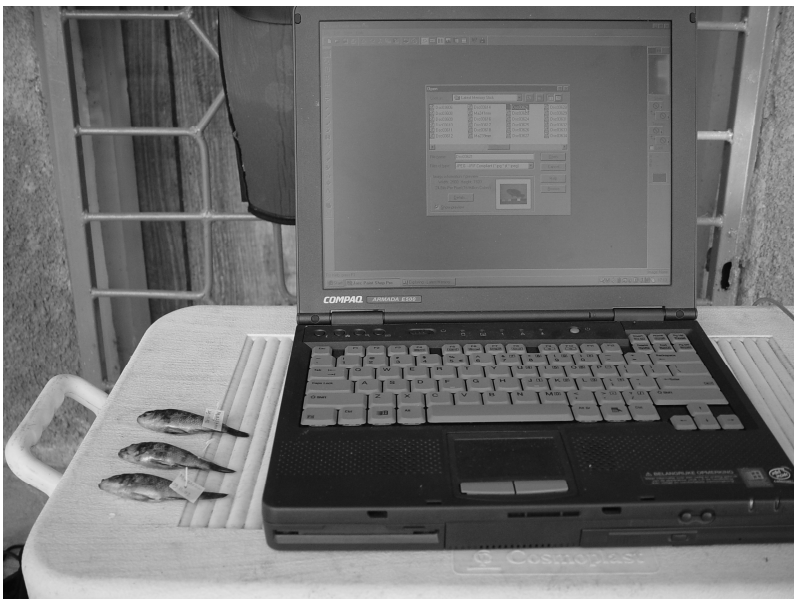
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Chapter 7

‘Characters are sometimes transferred to the offspring at an earlier age than that at which they first appeared in the parents, so that the parents may have varied when adult, and have transferred their characters to their offspring whilst young.’

Darwin, 1871



Heritability and heterochrony of polychromatism in a Lake Victoria cichlid fish: stepping stones for speciation?

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In many haplochromine cichlid fish, male nuptial coloration is subject to female mate choice and plays a central role in the evolution of reproductive isolation between incipient species. Intraspecific variation in male coloration may serve as a target for diversifying sexual selection and provide a starting point for species divergence. Here, we investigated a polychromatism in *Neochromis omnicaeruleus*, a haplochromine from Lake Victoria, East-Africa. In this species, male coloration ranges from skyblue to yellow-red and females are grey-blue to yellow. We found that both genetic and environmental factors influence the expression of these colours during individual development. In a natural population, we found that male colour was associated with size and sexual maturity: yellow males were smaller than blue males and tended to be sexually immature. In females, size and maturity did not differ between colour types. Laboratory crosses revealed that there is a heritable component to the observed colour variation: yellow parents produced more yellow offspring than blue parents. Together with repeated aquarium observations of yellow individuals that gradually become blue, these data suggest that yellow males change to blue as they approach sexual maturity, and that the timing and occurrence of this transition is influenced by both environmental and genetic effects. The significance of this mechanism of colour expression as a possible target for divergent selection remains to be evaluated.

Introduction

The haplochromine cichlids of the East-African Lakes Malawi and Victoria are well-known for their astounding species diversity, which has evolved in relatively little time (Kocher 2004). Consequently, evolutionary biologists have embraced them as model systems for speciation research (Turner & Burrows 1995; Galis & Metz 1998; Van Doorn et al. 1998; Kornfield & Smith 2000; Seehausen 2000; Danley & Kocher 2001; Kocher 2004). Because of their bright colours, haplochromines are similarly popular with aquarists worldwide (see www.nvcweb.nl, www.cichlids.com, www.cichlidae.com).

This colour variation plays a central role in the evolution and maintenance of haplochromine species richness. First, closely related haplochromines tend to be morphologically similar but strikingly different in male nuptial coloration (Albertson et al. 1999; Seehausen & Van Alphen 1999; Allender et al. 2003). Second, haplochromine colour patterns can determine mate choice that maintains reproductive isolation between closely related species (Knight et al. 1998; Seehausen & Van Alphen 1998). Third, female mate choice exerts directional sexual selection on male coloration within species from Lake Malawi (Pauers et al. 2004) and Lake Victoria (Chapter 2). Fourth, in a sibling species pair from Lake Victoria, Haesler & Seehausen (2005) demonstrated that both male coloration and female preference have a genetic basis. Finally, the intraspecific colour polymorphisms that are common among haplochromines resemble the colour differences that exist between species (Seehausen et al. 1999c). Together, these observations suggest that female preferences may exert divergent sexual selection on male colour patterns, which may accelerate or even drive the process of speciation.

By definition, speciation by sexual selection requires variation in mate preferences and preferred traits *within* species. Here we investigate the colour variation in both sexes of the Lake Victoria cichlid *Neochromis omnicaeruleus*. In this species, three distinct colour morphs have been described previously: two blotched morphs and one 'plain', presumably ancestral morph (Seehausen & Van Alphen 1999). Field observations show that among individuals of the plain morph, there is considerable colour variation, ranging from skyblue to bright yellow with red dorsum and chest in males, and from grey-blue to bright yellow in females (Seehausen 1996). In the present study, we quantify the relative abundance of these colours in a natural population. To assess the potential for assortative mating between colour types, we measure size and sexual maturity of all individuals caught. We also quantify the depth distributions of each colour type, because this may reflect adaptations to different light environments. In the turbid Lake Victoria waters, the ambient light intensity rapidly decreases with depth and the spectrum shifts towards longer wavelengths (De Beer 1989). Consequently, if body coloration is subject to sexual selection for conspicuousness, blue individuals should abound in shallow water and yellow ones in deeper water. Finally, to assess the role of *N. omnicaeruleus* colour variation as a possible target for selection, we carry

out a laboratory crossing experiment that explores the genetic basis of the observed variation.

Methods

Fish

Like all other haplochromine cichlids, *Neochromis omnicaeruleus* is a female mouthbrooder. Adult males guard spawning territories on the rock bottom that they defend against hetero- and conspecific intruders. Males attract females with vigorous courtship displays. After spawning, females mouthbrood the fry for about three weeks. We studied a population at Makobe Island in the western Speke Gulf (Tanzania), where the water is relatively clear (Secchi reading in the study period: $\text{mean} \pm \text{se} = 223 \pm 9.5$ cm [43 measurements]). At this island, *N. omnicaeruleus* occurs at water depths between 1 and 7 meters and is most abundant around 2 meters depth (Seehausen & Bouton 1997). Three distinct colour morphs occur in both sexes: white blotched, orange blotched and 'plain'; several intermediate phenotypes occur but are rare (Seehausen & Van Alphen 1999). We focus on colour variation within the plain, presumably ancestral morph. In males, about 99% of all individuals are plain (Seehausen & Van Alphen 1999). Coloration ranges from metallic blue to bright yellow (see Figure 7.1), with blue males being most abundant among territorial males (Seehausen 1996; this paper). Yellow and intermediately coloured males often are red on the dorsum, dorsal fin and chest. About half of all females are plain (Seehausen & Van Alphen 1999). Coloration of plain females ranges from grey-blue to bright yellow. Both sexes express 5-7 dark vertical bars. We use the suffixes GB, B, Y and G to denote grey-blue, blue, yellow and green fish.

Field sampling

In 12 days of sampling, spread evenly over a time period between November 2002 and April 2003, we collected 274 males (2 blotched and 272 plain) and 216 females (104 blotched and 112 plain), using gillnets with stretched mesh sizes of 10.5, 12.5, 16.5 and 18.5 mm. We set all nets at three depth ranges: between 1 and 2 metres (sampling effort: 170 minutes in 3 days, 307 individuals collected), between 3 and 4 metres (280 minutes in 4 days, 105 individuals) and between 5 and 6 metres (320 minutes in 5 days, 78 individuals). Because *N. omnicaeruleus* is most abundant in shallow water, catching effort in deeper water had to be high to obtain reasonable sample sizes. All fish were photographed immediately after capture in a plexiglass cuvette, using a digital camera (Sony DSC-F707), and subsequently put on melting ice. White balance of photographs was adjusted in PhotoShop (6.0, Adobe Systems Inc.) using a Kodak colour strip that was attached to the cuvette (No. Q-13, Eastman Kodak, Rochester, NY). Coloration of the plain morphs was scored by eye off the digital images, using the colour strip for reference. We scored 100 females as either grey-blue or yellow. Males (268 individuals) were scored in three categories: blue, yellow or green. Fish were subsequently

measured (standard length SL, to the nearest 0.1 mm; 99 females and 264 males) and dissected to determine gonadal maturation on a scale from 1 to 5 (GS; 97 females, 252 males). Gonads mature gradually with age; individuals scored as 4 and 5 are ready to spawn. In females, size at 50% maturity=63 mm 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). In males, size at 50% maturity is 86 mm (Seehausen et al. 1998a).

Laboratory crosses

Parental fish were collected from Makobe Island in February 2001 and in February 2003, using gillnets and hook and line. They were brought to the aquarium facilities at Leiden University (the Netherlands) and kept in either all-male or mixed groups (consisting of one male and several females) in 250-liter aquaria. Shelter was provided by PVC tubes and the bottom of the tanks was covered with a 5 cm layer of gravel. Water was continuously filtered; temperature was kept at $24\pm 2^{\circ}\text{C}$ and a 12:12h light:dark cycle was maintained. Fish were fed commercial pellets (2 times a week) and a mixture of shrimps and green peas (4 times a week). A microchip was inserted into the abdominal cavity of each individual for identification (12 mm glass tags, type UKID122GL, Biomark Inc., Idaho, USA). Brooding females were removed from the tanks between 3 and 10 days after spawning and housed individually in 3-liter tanks. One to two weeks after the first release of their fry, females were moved to a tank with a male of the other colour than the one they previously spawned with. In this way, we tried to have as many females as possible mated with both yellow and blue males. The eventual crossing scheme involved seven females (four yellow, three grey-blue) and four males (two yellow, two blue) (see Table 7.2). Three females mated with males of both types, all three spawned with a yellow male first (this is mainly due to the fact that the number of available yellow males decreased with time, see discussion). All spawnings occurred between April and October 2003. Colour and sex of offspring were scored about 1 year after spawning (mean $\pm$ se=359.5 $\pm$ 3.3 days; n =310 offspring; in the laboratory, *N. omnicaruleus* reaches sexual maturity at an age of about 8 months). While scoring the offspring, the colours of the parental fish were not known to the observer. To facilitate data analysis, both male and female colour was scored by eye in two categories: blue or yellow in males, grey-blue or yellow in females. Mortality, monitored from the moment the fry were released from the mouth of their mother, was below 10% for all clutches. We did not find evidence for sex-specific mortality in the experimental clutches: sex-specific mortality could lead to correlations between sex ratio and clutch size and/or age at scoring, but these correlations were absent (n =10 clutches; Pearson's r =0.25, p =0.48 and r =0.29, p =0.41, respectively). Whereas this result may be due to our small sample size and the small variation in age at scoring, the low overall mortality brings about that any effect of sex-specific mortality will be small.

Data analysis

The depth distributions of colour morphs were compared in χ^2 tests. To estimate the depth of maximum abundance for each colour type within the samples range of 1-6 m, data were adjusted for sampling effort at different depths. Standard length was normally distributed and analysed using independent samples t-tests; gonadal stages were compared using Mann-Whitney U tests. These statistics were done in SPSS (10.0, 2000, SPSS Inc.). The relationship between standard length and gonadal maturity stage was analysed by fitting generalised linear models (GLMs) with Poisson distributions. For the laboratory crosses, we analysed the effects of the identity and colour of the parental fish on the numbers of sons and daughters of different colours, by fitting GLMs with binomial distributions. Sex ratio was analysed in the same way. Clutch size was analysed using GLMs with a Poisson distribution. All GLM analyses were done using R software (Ihaka & Gentleman 1996; <http://www.r-project.org>); significance levels were determined by F-tests.

Results

Field sampling

We colour-scored 100 plain females (65 grey-blue; 35 yellow) and 267 males (138 blue; 94 green; 35 yellow). The depth distribution did not differ between colour morphs (females: $\chi^2=2.15$, $df=1$, $p=0.34$; males: $\chi^2=0.63$, $df=2$, $p=0.73$; Table 7.1). Consequently, the relative abundance of colour morphs did not change after correcting for catch effort at different depths (females: 35% yellow; males: 52% blue, 35% green, 13% yellow). Grey-blue and yellow females did not differ in size or gonadal maturity (SL: $n_{GB}=64$, $n_Y=35$; $t=0.34$, $p=0.73$; GS: $n_{GB}=63$, $n_Y=34$; MWU $Z=0.12$, $p=0.9$; Table 7.1, and Figure 7.1 on page 113). In males, there were significant differences between morphs: blue males were significantly larger than yellow males ($n_B=137$, $n_Y=34$; $t=6.37$, $p<0.001$) and than green males ($n_B=137$, $n_G=93$; $t=6.03$, $p<0.001$), and they had significantly higher gonadal maturity scores ($n_B=132$, $n_Y=33$; MWU $Z=2.91$, $p=0.004$; $n_B=132$, $n_G=87$; $Z=2.77$,

Table 7.1: Mean size (standard length, SL), mean water depth and the proportion of sexually mature (GS>3) individuals among grey-blue (GB), blue (B), green (G) and yellow (Y) males and females in the field sample.

	males			females	
	B ($n=138$)	G ($n=94$)	Y ($n=35$)	GB ($n=65$)	Y ($n=35$)
SL (mm; mean $\pm$ se)	74.7 $\pm$ 1.3	64.5 $\pm$ 1.1	62.2 $\pm$ 1.5	63.9 $\pm$ 1.2	64.6 $\pm$ 1.8
Proportion of sexually mature fish (GS>3)	20.5% (30/132)	4.6% (4/87)	3.0% (1/33)	11.1% (7/63)	5.9% (2/34)
estimated depth of max. abundance (m; mean $\pm$ se)	2.1 $\pm$ 0.1	2.2 $\pm$ 0.1	2.2 $\pm$ 0.2	2.0 $\pm$ 0.1	2.0 $\pm$ 0.2

$p=0.006$; Table 7.1 and Figure 7.1). Green and yellow males did not differ in SL nor GS ($p>0.17$). The differences in size and maturity between blue and green/yellow males were due to the near absence of large, mature yellow or green males (Figure 7.1). The relationship between size and gonadal stage did not differ between males of different colour morphs ($F_{1,249}=1.89$, $p=0.17$).

Laboratory crosses

The results of the crossing experiment are summarised in Table and Figure 7.2. The number of yellow males in a clutch differed significantly between mothers ($F_{6,3}=2.12$, $p=0.048$) and fathers ($F_{3,6}=3.18$, $p=0.023$). Mothers' colour had no effect ($F_{1,8}=0.13$, $p=0.72$), but there was a trend for yellow fathers to produce more yellow sons ($F_{1,8}=3.39$, $p=0.066$). The number of yellow females in a clutch was neither related to mother's identity ($F_{6,3}=1.29$, $p=0.26$) nor colour ($F_{1,8}=1.13$, $p=0.29$). There was a significant effect of father's identity ($F_{3,6}=6.23$, $p=0.0003$) and yellow fathers produced significantly more yellow daughters ($F_{1,2}=8.08$,

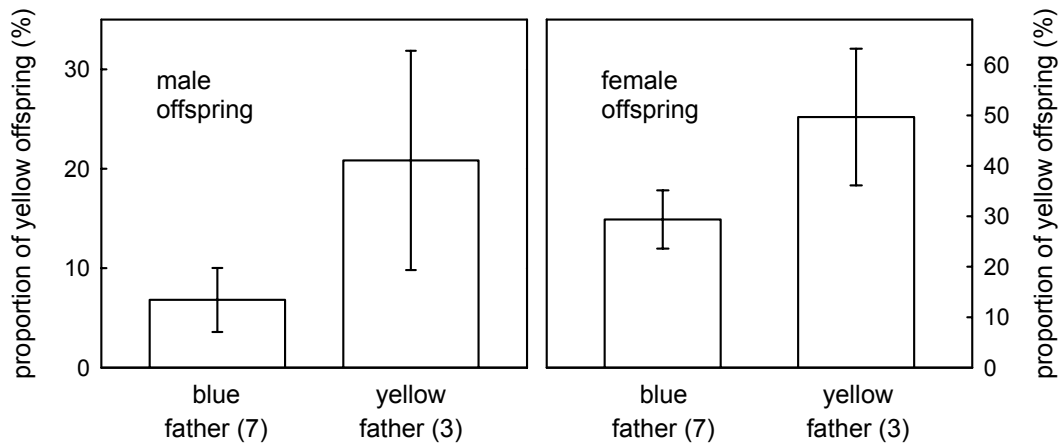


Figure 7.2 Results of the crossing experiment: the proportion of yellow individuals in the offspring of blue vs. yellow fathers. Left panel: male offspring; right panel: female offspring. Numbers in brackets indicate the number of clutches. Bars are means with standard errors.

Table 7.2 Results of the crossing experiment. B: blue; GB: grey-blue; Y: yellow.

mothers		fathers		male offspring			female offspring			clutch size	sex ratio (% males)
no.	colour	no.	colour	Y	B	Y %	Y	GB	Y %		
1	GB	1	Y	3	9	25	13	11	54.2	36	33.3
1	GB	2	B	3	10	23.1	1	10	9.1	24	54.2
2	Y	1	Y	0	10	0	8	25	24.2	43	23.3
2	Y	2	B	0	1	0	3	16	15.8	20	5
3	Y	4	Y	4	5	44.4	12	5	70.6	26	34.6
3	Y	3	B	1	10	9.1	5	22	18.5	38	28.9
4	GB	3	B	1	22	4.3	8	9	47.1	40	57.5
5	GB	3	B	1	8	11.1	10	16	38.5	35	25.7
6	Y	3	B	0	6	0	7	8	46.7	21	28.6
7	Y	3	B	0	6	0	6	14	30	26	23.1

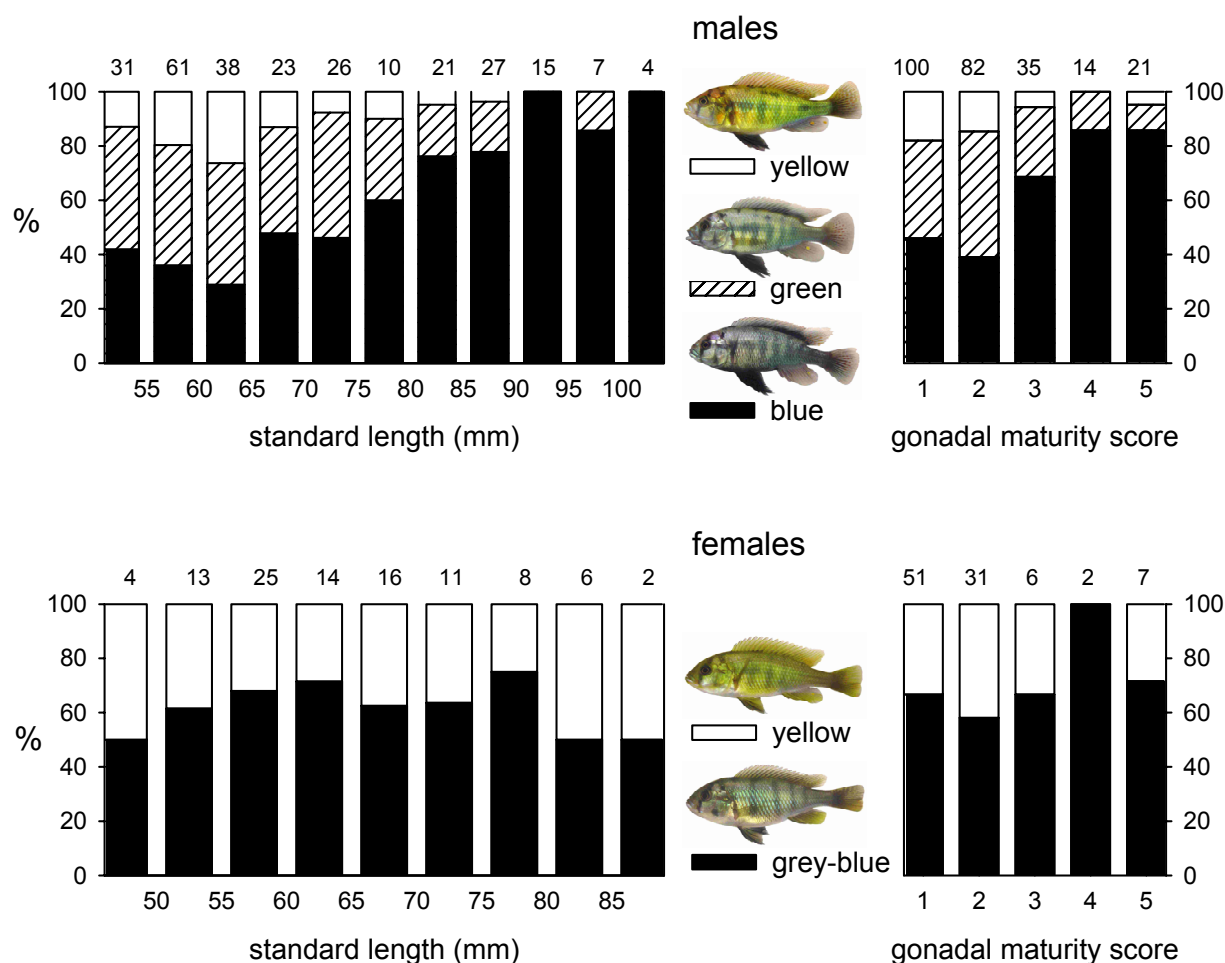


Figure 7.1 Frequency distributions for size (standard length) and gonadal maturity score of plain *N. omnicaeruleus* morphs at Makobe Island. Upper panel: males; lower panel: females. Numbers above the bars indicate sample sizes.

$p=0.0045$). The proportions of yellow sons and yellow daughters in a clutch were significantly correlated (Spearman $r_s=0.55$, $p=0.013$, $n=10$ clutches).

Offspring sex ratio ranged from 5 to 54% males (mean $\pm$ se 31.4 ± 4.8). Sex ratio was not affected by father's identity or colour (all $F < 0.59$, $p > 0.44$), but differed significantly between individual mothers ($F_{6,3}=3.68$, $p=0.001$) with yellow mothers producing lower sex ratios ($F_{1,8}=10.6$, $p=0.001$). This result remained significant after excluding the lowest sex-ratio produced (5%): $F_{1,7}=7.15$, $p=0.008$. Further analysis of the absolute numbers of sons and daughters revealed that yellow mothers did not produce more daughters than grey-blue females ($p=0.43$) but fewer sons ($F_{1,8}=11.73$, $p=0.0006$). The resulting difference in clutch size was not significant however (yellow mothers: 27.5 ± 5.3 , grey-blue mothers: 33.2 ± 2.7 , $F_{1,8}=1.74$, $p=0.19$). Clutch size differed significantly among fathers ($F_{3,6}=3.71$, $p=0.011$), but there was no relationship with father's colour ($F_{1,8}=2.28$, $p=0.13$).

There were no significant interaction effects between mother's colour and father's colour on either clutch size or sex ratio (all $F < 1.35$, $p > 0.26$). Offspring colour did not covary with clutch size or sex ratio (all $F < 2.6$, $p > 0.11$).

Discussion

Environmental induction of colour change during development

At Makobe Island, 35% of the plain *N. omnicaeruleus* females that we caught were yellow. Yellow and grey-blue females did not differ in size, gonadal maturity or depth distribution. In males, 13% of the plain individuals were yellow, 35% were green and 52% were blue. As in females, male colour types did not differ in depth distribution. These findings do not support the hypothesis that sexual selection for conspicuous males has lead to an increasing number of yellow males with greater depth. In fact, our results show that the potential for colour-based mating preferences is limited: among the males approaching sexual maturity (i.e. larger than 75 mm standard length or gonadal stage beyond 3), yellow and green males were rare (about 20%). Moreover, yellow territorial males were not seen during underwater observations (2000-2003, approximately 300 diving hours at 1-7 m depth). This suggests that either the yellow and green males die before reaching maturity, or, more likely, they become blue.

During the one-year period in which the clutches from the crossing experiment were kept, colour and sex were not scored. This was because fish could not be recognised individually, and because the regular occurrence of colour change was unknown at the start of this study. Throughout the study however, large numbers of *N. omnicaeruleus* were kept in our laboratory. In these fish, we observed several yellow males ($n > 20$) that changed to blue, typically coinciding with the onset of territory settlement and sexual activity. For example, one wild-caught yellow male became blue two weeks after introduction into an all-female tank. Other yellow males however remained yellow until death. The reversed change, from blue to yellow, was never observed, also not in males that moved from a dominant to a subdominant position or that were withheld physical or visual contact with females. During the colour switch from yellow to blue, several males ($n > 10$) passed through an intermediate phase of greenish coloration. This implies that the green males in the field sample represent an intermediate stage. The field data support this: the average size and gonadal stage of green males were intermediate between blue and yellow (Table 7.1).

Laboratory observations further indicate that not all males start yellow. Males may be blue as soon as it is possible to sex them - sometimes already at 2 cm standard length. In the laboratory crosses, the majority of male offspring were blue at the time of scoring (87%). Some of these may have gone through a colour switch before they were scored, because they were sexually mature at that time (*N. omnicaeruleus*, like most haplochromines, mature at a smaller size in the laboratory than in the field: ~6 cm instead of ~8 cm).

The field data did not show any evidence for a colour switch in females. However, laboratory observations show that females can also undergo colour change: after housing a large, brightly yellow, female with a group of smaller females, she turned completely grey-blue within two weeks. This happened after she obtained a dominant position and performed male-like behaviour, defending

a territory and even courting the other females. Dissection later confirmed that she was a female. Similar observations were made several times while breeding *N. omnicaeruleus* in the laboratory (OS pers. obs.). These observations suggest that both sexes may be liable to colour change, and that similar environmental conditions may affect this change. In nature, females may not experience these conditions, but the physiological mechanism may be present in both sexes.

Heritability of colour expression

The crossing experiment strongly suggests that there is a heritable component to the observed colour variation. This component may either influence coloration directly, or determine the occurrence and timing of a colour change from yellow to blue. Dietary differences cannot explain the variation in colour expression, because the laboratory-bred fish were fed a standard diet and yet expressed the same spectrum of colour variation as observed in the lake. Moreover, we would expect dietary differentiation to be reflected in differences in microhabitat distribution in the field (Bouton et al. 1997), for which we found no indication. We therefore conclude that genetic factors influence colour expression in the plain morph of *N. omnicaeruleus*.

The blotch polymorphism that occurs in *N. omnicaeruleus* (as well as in other haplochromines) is strongly associated with sex determining genes (Seehausen et al. 1999b), causing female-biased sex ratios in certain crosses. We did not find clear evidence for similar effects of the blue-yellow polychromatism: offspring sex ratio and coloration were not correlated and there were no interaction effects between parents' colour and offspring sex ratio. However, yellow females produced fewer sons than grey-blue females, resulting in lower sex-ratios. This could be due to lower viability of male offspring from yellow mothers, which would explain the absence of a correlation between the colours of mothers and sons. Yet, yellow mothers did not produce significantly smaller clutches. This indicates that larger sample sizes are required to evaluate the relationship between the blue-yellow polychromatism and sex determination.

Hormonal regulation of heterochrony

Heterochrony, i.e. a change in the relative timing of developmental events in evolution (Smith 2003), can create radically different phenotypes within species. These changes may come about through changes in hormone levels or in sensitive periods (e.g. in insects: Nijhout 1999). Developmental colour changes occur in several cichlid species, but little is known about the underlying mechanisms. For example, in the polychromatic Midas cichlid from Nicaragua (*Amphilophus citrinellum*), adult coloration is heritable, but the gradual change from the 'normal' to the 'gold' phenotype takes place at different times in different individuals (Barlow et al. 1990). Endocrine regulation of colour expression has been well studied in the side-blotched lizard *Uta stansburiana*. In this species, yellow-throated males behave as non-territorial sneakers. In the course of the breeding season however, some males gradually develop a blue patch on the throat and change to a territorial

strategy (Sinervo et al. 2000). This transformation is associated with an increase in testosterone levels in the blood (Sinervo et al. 2000). However, whereas alternative mating tactics occur in several cichlid species (Taborsky 1994), it is unlikely that the yellow *N. omnicaeruleus* males are sneakers: yellow males with mature testes were rare in our field sample (Figure 7.1) and the relationship between size and gonadal maturity stage did not differ between male colour morphs. Moreover, sneaking was never observed during underwater observations of territorial males in the lake (but our ability to detect it was limited: we observed only two spawnings during ~50 hours of observation; unpublished data). Also in other haplochromine species, territory ownership is a prerequisite for reproductive success and sneaking strategies appear unimportant (Chan & Ribbink 1990; Chapter 2).

Functional significance

High levels of circulating androgens may entail costs in terms of immune suppression (Grossman 1985), increased metabolism (Ros et al. 2004) and social costs (Olsson 1994; Wikelski et al. 2005). Yellow *N. omnicaeruleus* males may postpone androgen upregulation to minimise these costs. For example, the effect of coloration on inter-male aggression and dominance is well documented in cichlids (e.g. Evans & Norris 1996; Barlow 1998; Dijkstra et al. 2005). Yellow males may resemble females more than do green or blue males, which would reduce the amount of aggression they experience from other males. This is consistent with the observation that yellow males apparently switch to blue as they approach sexual maturity, the point at which territory settlement becomes more important than aggression avoidance. The benefits of this strategy may be frequency-dependent, as increasing numbers of yellow males may lead to improved sex recognition or increased aggression to females by other males. The yellow strategy may entail costs as well: androgens affect muscle development and body size at maturity (Pullin 1991), which in cichlid fish may influence reproductive success either directly, through female preferences for male size (Rogers & Barlow 1991; Nelson 1995), or indirectly, through male-male competition and female preference for territory size or quality (McKaye et al. 1990; Nelson 1995; Kellogg et al. 2000; Chapter 2). The relative importance of the costs and benefits associated with androgen regulation may depend on a suite of environmental variables such as food availability, differential predation, population density and the relative abundance of the different strategies. Fluctuations in these variables may help to maintain genetic variation in natural populations.

The red coloration on the dorsum, dorsal fin and chest of yellow males has never been observed in females. These red patches have the appearance of sexually selected ornaments, which seems to conflict with the observation that yellow males tend to be sexually immature. Moreover, if aggression avoidance through female resemblance is a major selective pressure, red coloration seems maladaptive. Possibly, the red coloration interferes with species recognition and thereby reduces the level of intraspecific aggression (Seehausen & Schluter 2004).

The adaptive value of colour variation in females remains unclear. Female coloration was unrelated to body size or sexual maturity and differential aggression towards grey-blue or yellow females seems unlikely, suggesting that female coloration may be selectively neutral.

Implications for speciation

Developmental plasticity and heterochrony of the cichlid feeding apparatus (e.g. Meyer 1987; Witte et al. 1990) have been implicated to either constrain (Meyer 1987) or promote (Wimberger 1994) species divergence (Stauffer & Gray 2004). Because haplochromine colour patterns mediate inter- and intraspecific mate choice, and because the blue-yellow polychromatism of *N. omnicaeruleus* resembles the colour variation present among other haplochromine species, it may provide a starting point for divergent selection by mate choice. Currently however, the number of yellow, sexually mature males may be too low for females to develop mating preferences for them. Moreover, our data suggest that among the reproductively active blue males, there is considerable variation in their colour switch history, which seems impossible for choosy females to select upon. In a similar fashion, 'gold' morphs of the Midas cichlid can reproduce before developing the 'gold' phenotype, precluding the evolution of reproductive isolation (Barlow 1998).

Conclusion

This study represents the first investigation of blue-yellow polychromatism in *N. omnicaeruleus*. We show that both environmental and genetic factors determine colour expression and/or development. These results may be of general importance in the study of haplochromine colour variation, because similar colour changes have been observed also in other species from Lake Victoria (Florin 1991; F. Witte pers. obs., OS pers. obs.). The significance of this colour variation as a possible target for divergent selection remains to be evaluated. Specifically, the proximate determinants (social environment, gonadal maturation, endocrine regulation, genetics) and ultimate selection pressures (inter-male aggression, female choice, predation) on male and female coloration require further study.

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