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Sexual selection and speciation: mechanisms in Lake Victoria cichlid fish

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Sexual selection and speciation
Mechanisms in Lake Victoria cichlid fish

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Sexual selection and speciation: mechanisms in Lake Victoria cichlid fish

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Cover: detail of a so-called 'Tingatinga' painting by the Dar es Salaam artist Haji. This well-known Tanzanian style of painting, named after its originator Edward Tingatinga (1932-1972), is characterised by the use of bright, undiluted colours and traditionally features large, bold images of animals and birds on a monotone background. Nowadays artists also depict Tanzanian landscapes and scenes from everyday life, sometimes referring to contemporary events and social issues. The style has been commercially driven since its inception: even the largest canvases are produced within a few days and most painters work limited variations around favourite subjects. Tingatinga should not be considered traditional African art, some have tagged it Africa's answer to Pop Art.

Sexual selection and speciation
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Chapter 1

'All structures and instincts by the aid of which the male conquers other males, and by which he allures or excites the female, will be fully discussed, as these are in many ways the most interesting.'

Darwin, 1871

General Introduction

Natural and Sexual Selection in Animal Speciation

Speciation, the process by which new species arise, is one of the most tenacious biological problems. One reason for this is that speciation proceeds relatively slowly, hindering observation and hypothesis testing in natural populations. Consequently, speciation research has long focused on describing patterns of species diversity rather than speciation itself, and for many years has been the domain of systematists. Currently, there is increasing interest in the process of speciation itself (e.g. Howard & Berlocher 1997; Schilthuizen 2001; Coyne & Orr 2004; Dieckmann et al. 2004; Gavrilets 2004; and special issues of TREE [Vol. 16 Iss. 7, 2001] and PNAS [Vol.102 Iss 15, 2005]). Whereas theoretical papers amply outnumber experimental ones (Kirkpatrick & Ravigne 2002), experimental studies in natural populations are required to test the assumptions of theoretical models, quantify selection pressures and assess the likelihood and relative importance of alternative theoretical scenarios (Turelli et al. 2001). Moreover, empirical studies typically drive the development of theory, as they discover patterns that set the conceptual framework for theoretical analysis.

Besides the practical difficulties associated with the study of speciation in nature, progress has been hampered by confusion regarding several central concepts. For example, differences in emphasis have yielded many different definitions of 'species' and 'speciation' (Hey 2001). Likewise, the traditional distinction between allopatric and sympatric speciation has proved to be sometimes unpractical. Most speciation will indeed be 'parapatric', characterised by incomplete interruption of gene flow between incipient species (e.g. Gavrilets 2000).

Rather than aiming for a list of unambiguous definitions to describe the endpoints of evolution, evolutionary biologists increasingly focus on the processes that cause diversification. These can be roughly divided into two categories: random processes (genetic drift, founder effects) and divergent selection. Classic views of speciation (Dobzhansky 1937; Mayr 1942) have emphasised the former: geographically isolated populations evolve along different paths and given enough time before secondary contact, interbreeding will not occur. This is because behavioural or physiological aspects of mating systems have become incompatible (e.g. Mayr 1963; Templeton 1981), or because interbreeding results in sterile or inviable offspring, leading to the evolution of divergent mating preferences through reproductive character displacement or reinforcement (e.g. see Brown & Wilson 1956; Grant 1972; Liou & Price 1994; Servedio & Noor 2003).

The involvement of natural and sexual selection in speciation is increasingly supported by both theoretical and empirical studies (Kirkpatrick & Ravigne 2002). With or without geographical barriers, a heterogeneous environment may select for ecological specialisation and assortative mating, thus reducing gene flow between subpopulations and eventually leading to a level of reproductive isolation that is sufficient for the build-up and maintenance of genetic differences between

them (i.e. ecological speciation, for reviews see Schluter 2001; Rundle & Nosil 2005; for theoretical treatments see e.g. Fisher 1930; Lande 1982; Kirkpatrick 2001; Gavrilets 2004). A classic example is the threespine stickleback that has repeatedly evolved into limnetic and benthic morphs (Rundle et al. 2000). This process may also explain several cases of 'adaptive radiation': the rapid evolution of species diversity after colonisation of a novel habitat, such as remote islands or newly formed lakes (Schluter 2000). Textbook examples of such radiations include East-African cichlid fish, Hawaiian Drosophila's and the Galápagos finches. Also in full sympatry, ecological speciation may be feasible (Dieckmann & Doebeli 1999; Kondrashov & Kondrashov 1999; Fry 2003): resource competition among conspecifics can lead to disruptive natural selection that favours individuals utilising niches that are less crowded. This mechanism may explain host race formation in herbivorous insects (reviewed in Via 2001).

The theory of ecological speciation by divergent natural selection predicts that recently diverged species differ especially in traits associated with resource utilisation such as habitat use, diet and/or feeding morphology. The theory further predicts that within the niches of the parental species, hybrids with intermediate phenotypes are selected against: if ecological selection has driven the parental species apart, each species should be less well adapted to the niche of the other species, and hybrids should do intermediately well in either parental niche. As a consequence, if no other niches are available, hybrids should have lower fitness than either parental type and assortative mating is favoured by selection. Both these predictions may be upheld in the Darwin's finches (Grant & Grant 1993) and the threespine stickleback (Rundle & Schluter 1998). Hence, adaptive radiation in these groups may be driven by ecological speciation through natural selection.

In other taxa however, recently diverged species show only slight ecological differentiation but have diverged considerably in traits related to mating behaviour and sexual selection. This is the case in the radiations of Hawaiian Drosophila's and East-African haplochromine cichlids (Dominey 1984), in which closely related species are ecologically similar but differ in chemical, acoustic and behavioural courtship signals (Drosophila's: Boake 2005) or male nuptial coloration (cichlids: McElroy et al. 1991; Albertson et al. 1999; Seehausen & Van Alphen 1999; Allender et al. 2003). Also in other taxa, there are several examples of subpopulations or incipient species that differ in secondary sexual traits and little else: jumping spiders in Arizona (Masta & Maddison 2002), Anolis lizards in Puerto Rico (Leal & Fleishman 2004), and bowerbirds in Indonesia (Uy & Borgia 2000). These observations lead to the hypothesis that speciation could be the consequence of divergent sexual selection (West-Eberhard 1983; Dominey 1984; Coyne 1992).

In several of these cases however, directional or disruptive sexual selection has not been demonstrated. Moreover, quantitative comparison of divergence along two different axes of adaptation, one driven by ecological selection and the other by sexual selection, requires understanding of the genetic mechanisms that

underlie these adaptations. Such insights are only beginning to emerge (Price 2002; Albertson et al. 2003; Streelman et al. 2003; Haesler & Seehausen 2005). Nevertheless, the striking discrepancy between divergence in secondary sexual characters and ecologically relevant characters in many of these groups strongly suggests that sexual rather than ecological selection has driven population divergence. Speciation may occur when mating preferences and preferred traits have become so different that they lead to reproductive isolation. This may happen because individuals of different populations do not recognise each other anymore as conspecifics and therefore do not mate, or because hybrid phenotypes have lower fitness and therefore selection favours assortative mating. These scenarios have inspired the prediction that taxa characterised by strong sexual selection, e.g. highly polygamous lek breeders, should be relatively species-rich. This idea was already postulated by Darwin (1871) and several comparative phylogenetic studies have found affirmative evidence for this (e.g. haplochromine cichlids: Seehausen et al. 1999a; insects: Arnqvist et al. 2000; Agamid lizards: Stuart-Fox & Owens 2003), but sometimes the results are ambiguous (for example in birds: Barraclough et al. 1995; Møller & Cuervo 1998; Owens et al. 1999; versus Price 1998; Morrow et al. 2003), or they implicate ecological rather than sexual selection (Gage et al. 2002).

From a theoretical point of view, mate choice can establish reproductive isolation very rapidly, since it directly influences mating structure and does not require reinforcement through selection against hybrids (Kirkpatrick & Ravigne 2002). However, whereas theoretical models often assume that populations harbour genetic variation for mate preferences (Turner & Burrows 1995; Higashi et al. 1999; Takimoto et al. 2000), the empirical evidence for such variation is limited (Jennions & Petrie 1997; Widemo & Saether 1999; Arnegard & Kondrashov 2004). Extrapolation of the results of speciation models to natural systems is further hampered by the frequent assumption that female preferences are selectively neutral (or entail a small cost) and do not enhance offspring fitness (e.g. Lande 1981; Turner & Burrows 1995; Van Doorn et al. 2004; for reviews see Panhuis et al. 2001; Turelli et al. 2001). This assumption allows evolution of preference and trait in many directions and facilitates divergence, but it is opposed by numerous studies describing sexually selected traits that reliably indicate individual quality (for reviews, see Andersson 1994; Candolin 2003; Neff & Pitcher 2005). Female preferences for males that provide genetic quality or direct benefits are subject to natural selection and may not diverge so easily (Kirkpatrick & Nuismer 2004; but see Lorch et al. 2003; Edelaar et al. 2004; Reinhold 2004). Therefore, to understand the role of sexual selection in speciation we must identify the selection pressures acting on mating preferences.

In this thesis, I investigate the selection pressures acting on mating preferences and preferred traits in East-African cichlid fish. Before I further discuss the aims and scope of the thesis, I will first introduce the model system that I studied: the haplochromine cichlids of Lake Victoria.

East-African cichlid fish

The Cichlidae (Teleostei, Perciformes) constitute the most species-rich family of vertebrates, comprising more than 2000 species. They inhabit rivers and lakes in South and Central America and in Africa, with a few species occurring in Madagascar and Asia. The majority of species is found in the three Great Lakes of East-Africa: Tanganyika, Malawi and Victoria (Table 1.1, Figure 1.1). Within each of these lakes, cichlid fish have radiated into hundreds of species, making them important model systems for the study of explosive speciation and adaptive radiation (Kornfield & Smith 2000; Kocher 2004).

Lake Tanganyika is by far the oldest of the three lakes (9-12 million years, Cohen et al. 1993) and it harbours a less species rich but phylogenetically and phenotypically more diverse cichlid fauna than the other two (Fryer & Iles 1972). For example, in contrast to the endemic cichlids of Lakes Malawi and Victoria, its ~250 species (Snoeks et al. 1994) represent many different breeding styles: there are maternal and biparental mouthbrooders and substrate spawners, and polygamous, monogamous and co-operatively breeding species. They also show a large variation in body sizes, both the world's smallest and largest cichlids are Tanganyikan endemics: *Neolamprologus neofasciatus* (4 cm) and *Boulengerochromis microlepis* (70 cm).

Lakes Malawi and Victoria are much younger than Lake Tanganyika and their endemic cichlid faunas are less diverse. Besides a few Tilapiine species, all cichlids in Lakes Malawi and Victoria belong to the 'haplochromines', a monophyletic lineage of polygamous, mouthbrooding cichlids that is widely distributed across Africa. Several haplochromine species also occur in African rivers. Many lacustrine haplochromine species are rock-dwellers, restricted to the shallow waters of rocky shores (Ribbink et al. 1983; Seehausen 1996; Genner & Turner 2005). Local fishermen refer to these as *Mbipi* (Lake Victoria) and *Mbuna* (Lake Malawi).

Lake Malawi was formed about 4-5 million years ago, but it has dried out repeatedly (Delvaux 1995). Its ~500 endemic haplochromine species (Genner et al. 2004b) may have evolved within a very short time (Won et al. 2005). Similar to Lake Tanganyika, Lake Malawi is a very deep and narrow trough with clear wa-

Table 1.1 Physical and biological characteristics of the three Great East-African Lakes.

	Lake Tanganyika	Lake Malawi	Lake Victoria
age (my)	9-12	4-5	0.25-0.75
maximum depth (m)	1470	704	93
surface area (km^2)	32.600	30.800	68.600
maximum transparency (Secchi disc, m)	22	17	8.2[1]
minimum and maximum estimates of the number of endemic cichlid species	162-250	451-1000	447-1000

[1]Maximum Secchi disk readings in the study area of this thesis in 2000-2003: Mwanza Gulf 1.6 m; Speke Gulf 4.0 m (Maan et al., unpublished).

ters. In contrast, Lake Victoria is a less clear, shallow, saucer-shaped lake. Its basin formed about 0.25-0.75 my ago (Fryer & Iles 1972), but just like Lake Malawi it has dried out several times and probably filled up again as recently as 14.600 years ago (Johnson et al. 1996; Martens 1997; Johnson et al. 2000). Yet, the lake contains a minimum of ~500 haplochromine species (Seehausen et al. 1997b; Genner et al. 2004a), that apparently evolved very rapidly within the confines of the lake basin.

Haplochromine speciation

The precise temporal and spatial scales of haplochromine evolution, and the phylogeographic history of the Lake Victoria radiation in particular, are the subject of ongoing revision and discussion (Nagl et al. 2000; Fryer 2001; Seehausen 2002; Seehausen et al. 2003; Verheyen et al. 2003; Fryer 2004; Stager et al. 2004; Verheyen et al. 2004; Salzburger et al. 2005). There is general consensus however that speciation rates of lacustrine haplochromines have been exceptionally high (Kocher 2004; Won et al. 2005). The rivers and satellite lakes surrounding Lake Victoria do not contain many haplochromine species and only few of those are shared between these habitats and the main lake. It therefore appears unlikely that the hundreds of species now endemic in the lake have evolved in complete geographical isolation in different water bodies (Seehausen 2002). Nevertheless, some data indicate that the genetic diversity contained in the present species flock may originate from more than one ancestral species, that colonised the lake from the surrounding rivers and lakes (Nagl et al. 2000; Seehausen et al. 2003; Seehausen 2004; Salzburger et al. 2005). These ancestors subsequently radiated into a great diversity of species that differ in behaviour, ecology, morphology and coloration (Danley & Kocher 2001; Streelman & Danley 2003).

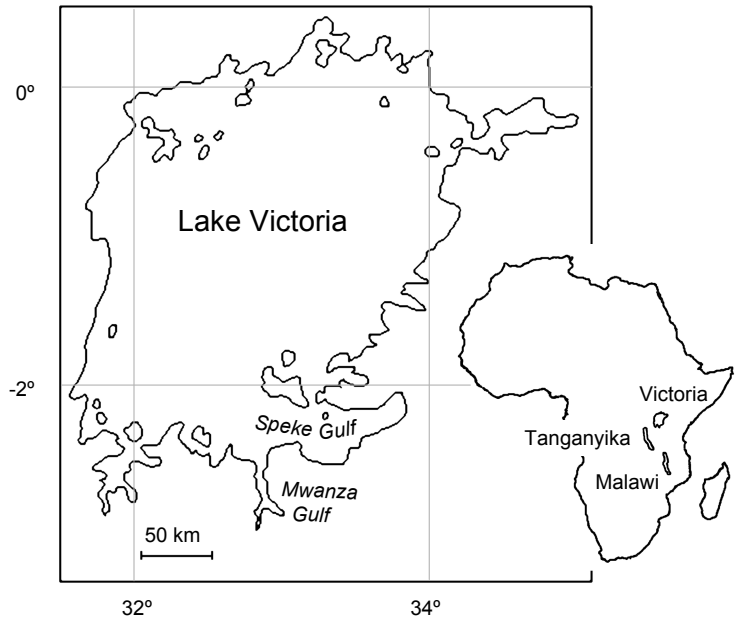


Figure 1.1 The three Great Lakes of East-Africa.

The flexible and versatile pharyngeal jaw apparatus and general anatomy of cichlids have probably contributed to this diversification, because functional versatility facilitates rapid adaptation to different ecological niches (Liem 1973; Galis & Drucker 1996). As mentioned earlier however, recently diverged sister species show very little divergence in trophic morphology, but rather tend to differ profoundly in male nuptial coloration (Albertson et al. 1999; Seehausen & Van Alphen 1999; Allender et al. 2003) or female coloration (Seehausen & Van Alphen 1999; Lande et al. 2001). The discrepancy between eco-morphological divergence and divergence in secondary sexual characters among haplochromines was already noted by Fryer & Iles (1972). Although sceptical about the importance of sexual selection, they interpreted haplochromine colour diversity as a way to facilitate mate recognition among co-occurring species otherwise similar in appearance.

Haplochromines are characterised by an extremely asymmetrical parental investment into offspring: whereas males contribute little more than sperm, females mouthbrood eggs and fry for several weeks, during which time they will not eat. This asymmetry is conducive of strong sexual selection by female mate choice (Andersson 1994). Together, these observations inspired the hypothesis that sexual selection is important in haplochromine speciation (Dominey 1984; McKaye 1991; Deutsch 1997).

Haplochromine colour variation

In Lakes Malawi and Victoria, two major patterns of colour variation exist among closely related sympatric haplochromines (Seehausen et al. 1999c). Most abundant in both lakes are the blue-red or blue-yellow polymorphisms or species pairs. Representatives are *Pundamilia pundamilia* and *Pundamilia nyererei* in Lake Victoria (Figure 5.1 on page 81) and members of the *Pseudotropheus* and *Aulonocara* genera in Lake Malawi (Konings 2001). Also common in both lakes are the blotch polymorphisms, characterised by black blotches on a white or orange background, with homozygous individuals being completely black, white or orange (Seehausen 1996; Konings 2001). The blotch polymorphism is associated with sex-determining genes (see Table 1 in Lande et al. 2001). In the context of speciation, blotch polymorphisms have mainly been studied in *Neochromis omnicaeruleus* (Lake Victoria, Figure 8.4 on page 128; Seehausen et al. 1999b) and *Maylandia zebra* (Lake Malawi, Knight 1999; Streelman et al. 2003).

Experimental studies have demonstrated that variation in these colours may mediate both interspecific (Seehausen & Van Alphen 1998; Knight & Turner 2004) and intraspecific (Seehausen et al. 1999b) mate choice. These findings confirm a central assumption of the hypothesis that divergent sexual selection on colour patterns contributes to haplochromine speciation: divergent colour pattern preferences can maintain reproductive isolation between incipient species, in the absence of intrinsic post-zygotic isolating mechanisms or geographical barriers to gene flow. Moreover, differences between species resemble polymorphisms observed within single populations of species, suggesting that the former may evolve

from the latter (Seehausen et al. 1999c). In this thesis, I aim to further evaluate this hypothesis. Specifically, I investigate the mechanisms that drive the evolution and divergence of mating preferences for colour patterns. Focusing on the two above-mentioned model systems from Lake Victoria: *Pundamilia* and *Neochromis*, I investigate the selection pressures acting on colour patterns and colour pattern preferences.

Sexual selection hypotheses and their importance for haplochromines

Numerous hypotheses have been contrived to explain mating preferences for particular traits (Andersson 1994) and the proposed mechanisms differ markedly in the extent to which they facilitate or constrain the evolution of preference variation (Lorch et al. 2003; Kirkpatrick & Nuismer 2004; Van Doorn & Weissing 2004). They can be roughly categorised as follows.

1. Sexual selection for direct benefits: the chosen sex provides parental care (e.g. threespine stickleback, Bell & Foster 1994), nutritious resources (e.g. crickets, Gwynne 2001), breeding substrate (e.g. shell-breeding cichlids of Lake Tanganyika, Sato 1994; and several bird species, Andersson 1994) or other direct benefits. Choosiness evolves in response to selection to maximise these benefits. In haplochromines, direct benefits may not be very important because, in most species, females are the more choosy sex and males do not provide care. This sexual selection hypothesis is therefore not explicitly addressed in this thesis. However, female choice may be influenced by direct benefits of territory quality because some territories may provide better shelter against predators or harbour fewer parasites than others. Similarly, a direct benefit of a preference for conditionally healthy, parasite-free mates could minimise the risk of transmission of infectious diseases or parasites. Male choosiness for direct benefits could lead to preferences for larger females (that may produce more eggs) or females in good condition (that may provide better care).

2. Sexual selection for genetic quality: the chosen sex provides 'good genes' that enhance the survival or reproductive success, and hence fitness, of the offspring. There are three, not mutually exclusive, ways that parental genes may increase offspring fitness:

    a) additive genetic effects on offspring survival or condition (e.g. preferences for older mates, Brooks & Kemp 2001). A special case is 'handicap selection' (Zahavi 1975). According to this mechanism, ornamental traits reduce individual survival, for instance because they require the investment of costly resources, or because they increase conspicuousness to predators. They are selected for by the choosy sex, because these expensive, 'handicapping' ornaments can only be afforded by high-quality individuals. The high-quality genes are conferred to the offspring.

b) additive genetic effects on offspring attractiveness, increasing offspring reproductive success (Fisherian runaway selection for arbitrary traits that make 'sexy sons', Fisher 1930).

c) non-additive compatibility effects (e.g. preferences for complementary sets of MHC alleles that improve offspring immune defence, as shown in the threespine stickleback, Aeschlimann et al. 2003). To some extent, the mate preferences exerted by both sexes of *Neochromis omnicaeruleus* fall into this category: they prefer mates that carry compatible alleles, which lead to even sex-ratios in the offspring (Seehausen et al. 1999b).

Each of these mechanisms may be important in haplochromine cichlids, and b) and c) are explicitly investigated in Chapters 3,4 and 8 of this thesis.

3. Sexual selection as a by-product of natural selection on sensory properties ('sensory bias' and 'sensory exploitation', Ryan 1990; Endler 1992). Sensory properties are selected to maximise the efficiency with which food resources and potential predators are detected. Such selection can result in sensory biases that affect the mating preferences of the choosy sex, and these can be exploited by the chosen sex. A textbook example is the mating call of the Tungara frog male, which efficiently exploits a pre-existing bias in the female auditory system (Ryan 1985).

   Seehausen et al. (1997a) suggested that ecological adaptations in the haplochromine visual system may affect mating preferences for colour patterns. In Chapter 5 of this thesis, I investigate this hypothesis.

Aims and scope of the thesis

With this thesis, I hope to contribute to a better understanding of the processes involved in haplochromine speciation. Specifically, I investigate the selective forces acting on colour traits and mate preferences for these traits in Lake Victoria haplochromines. The rationale behind this objective is as follows: whereas it is increasingly appreciated that the tremendous colour variation among haplochromine cichlids plays an important role in both inter- and intraspecific mate choice, very little is known about the processes that drive (or constrain) the evolution of these colour signals and colour preferences in the first place. As outlined above, sexual signalling may be subject to a variety of evolutionary pressures, exerting selection on both the senders and receivers of sexual signals. Insight in these selection pressures is required to assess the importance of haplochromine colours for the evolution of their extraordinary diversity.

I focus on two representative model systems: a pair of sibling species in the genus *Pundamilia* (Chapters 2-6) and the highly polymorphic species *Neochromis omnicaeruleus* (Chapters 7-8). In both laboratory and field conditions, I investigate the nature and strength of several possible determinants of haplochromine colour

signalling. I evaluate the consequences of my findings for the hypothesis that sexual selection by mate choice contributes to haplochromine speciation.

Thesis outline and summary of the chapters

Chapters 2-6: blue and red: the divergence of *Pundamilia pundamilia* and *Pundamilia nyererei*

Pundamilia pundamilia (Seehausen et al. 1998a) and *P. nyererei* (Witte-Maas & Witte 1985) are two very closely related species of Lake Victoria cichlids, that form reproductively isolated and ecologically differentiated sympatric sister species in some localities, but interbreeding colour morphs or incipient species in other localities. They represent a common pattern of colour variation among Lake Victoria haplochromine cichlids (Seehausen et al. 1999c). *Pundamilia pundamilia* males are metallic grey-blue and *Pundamilia nyererei* males are yellow laterally and bright red dorsally (see Figure 5.1, page 81). The two species are morphologically similar; the cryptically coloured females can be distinguished only with difficulty. Where water clarity permits colour signal transduction, the two species are differentiated, females have species-assortative mating preferences and use male coloration as a choice criterion (Seehausen & Van Alphen 1998). Within *P. nyererei*, male coloration is a more extreme red where water clarity and hence signal transduction is better. These observations lead to the hypothesis that sexual selection has been a driving force in the divergence of this species pair. This scenario rests on the premise that the phenotypic traits responsible for reproductive isolation between sister species evolve under sexual selection within species. However, interspecific differences in characters subject to mate choice do not necessarily signify that sexual selection was the cause of their divergence (Lande 1981; West-Eberhard 1983; Boake 2002). In **Chapter 2**, I tested this premise in *Pundamilia nyererei*. I studied a population at Makobe Island in the western Speke Gulf (Seehausen & Bouton 1997; Figure 6.1 on page 93), where the water is relatively clear. I carried out behavioural mate-choice experiments in the laboratory, field observations on territorial males in the lake, and a mesocosm experiment under semi-natural conditions, in which actual mating occurred. I found that male red coloration is subject to directional sexual selection by female mate choice: in both laboratory and field conditions, females responded more frequently to the courtship displays of brighter red males than to those of less bright males. This is consistent with the hypothesis that intraspecific sexual selection through female choice has driven the divergence in male coloration between *P. nyererei* and *P. pundamilia*. I also found that male territoriality is vital for male reproductive success, and that females tend to mate with more than one male for the fertilisation of a single clutch of eggs.

In Chapters 3 and 4, I investigated whether female preferences in *Pundamilia* may be driven by selection for 'good genes', and I assessed the potential for this selection to contribute to the divergence of *P. pundamilia* and *P. nyererei*. First (**Chapter 3**) I studied fitness correlates of male coloration in *Pundamilia nyererei*. As

demonstrated in Chapter 2, male red coloration in this species is subject to intraspecific sexual selection by female mate choice. Here, in the same field population, I found that variation in the extent and brightness of male coloration elements was not associated with variation in a set of strongly interrelated indicators of male dominance: male size, territory size and territory location. Instead, male territory size and coloration were independent predictors of male quality: female preferences for bright red males and males with large territories both selected against heavily parasitized males. Consistent with parasite-mediated sexual selection (Hamilton & Zuk 1982), males had higher and more variable parasite loads than females. I also showed that male coloration is carotenoid-based, illustrating the potential for honest signalling: carotenoids, while probably a limiting resource, are necessary for a variety of beneficial physiological functions, particularly in immune defence (Olson & Owens 1998; Hill 1999). Consequently, carotenoid displays are physiologically costly to produce and maintain, and thereby fulfil the requirements of an honest signal of individual quality (Zahavi 1975; Lozano 1994).

To investigate whether parasite-mediated sexual selection might have contributed to the divergence of female mating preferences and male colours between *P. pundamilia* and *P. nyererei*, I also studied the relationship between male nuptial coloration and parasite load in *P. pundamilia* from Makobe Island (**Chapter 4**). As described above, *P. pundamilia* males have metallic grey-blue bodies and dorsal fins, but their anal and caudal fins can be bright red. I found that this red coloration of *P. pundamilia* fins seems to be chemically similar to that in the body and fins of *P. nyererei*, but that it is not significantly associated with parasite load. Instead, I found that the blue coloration on the body and dorsal fin is negatively correlated with parasite load. I also found that parasite infestation rates differ quantitatively between the species, in a way that is consistent with species differences in diet and microhabitat. These results suggest that at an island where the sister species are genetically and ecologically well differentiated, parasite-mediated sexual selection within each species could cause divergent selection between the species on male coloration and parasite resistance. Hence, divergent sexual selection may not be inconsistent with 'good genes' models of sexual selection. I conclude that in populations where *P. pundamilia* and *P. nyererei* are ecologically differentiated, parasite-mediated sexual selection may strengthen reproductive isolation between the two species.

Divergent parasite-mediated sexual selection may not be enough to drive species divergence. This is because intermediate phenotypes are likely to experience intermediate parasite exposure rates and may therefore do well in intermediate habitats. As a result, assortative mating will not evolve. In **Chapter 5**, I therefore investigated another mechanism, that could have driven the divergent evolution of male coloration and female mating preferences between *P. pundamilia* and *P. nyererei*: divergent sensory drive. Several recent studies have presented evidence for divergent sensory drive in species that use visual signals in sexual communication (Boughman 2001; Fuller 2002; Leal & Fleishman 2004), indicating that it may be a potent driving force in speciation (Endler & McLellan 1988;

Boughman 2002). The hypothesis that sensory drive has been involved in the divergence of *P. pundamilia* and *P. nyererei* (Seehausen et al. 1997a) predicts that i) the photic environment in the microhabitat of the two species differs, ii) visual systems of the two species have diverged in adaptation to these different photic environments, and that iii) within a species, females prefer more conspicuous over less conspicuous males. As demonstrated in Chapter 2, the latter is the case for at least one of the two species: the red coloration of *P. nyererei* males is subject to directional sexual selection by female choice and the extent and brightness of red is negatively correlated with parasite load (Chapter 3). As demonstrated in Chapter 4, the extent of blue coloration in *P. pundamilia* males is negatively correlated with parasite load too. Here, I quantified the light environments of *P. pundamilia* and *P. nyererei* at Makobe Island and I demonstrated that they differ significantly: *P. nyererei*, which breeds in deeper water than *P. pundamilia*, inhabits a more red-shifted habitat. Whereas *P. nyererei* and *P. pundamilia* differ in visual properties at the molecular level (Carleton et al. 2005), it was unknown whether these species also differ in the perception of red and blue stimuli, in a way that could explain the divergent mating preferences of the two species. I used the optomotor response test to measure the context-independent behavioural responses to coloured light in both species. I found that the species differ in the predicted direction: *P. pundamilia* is more sensitive to blue light and *P. nyererei* is more sensitive to red light. I conclude that divergent sensory drive in a heterogeneous environment may have driven the divergence of *P. pundamilia* and *P. nyererei*. The fully sympatric distribution of the two species suggests that this process occurred without geographical isolation. Currently, the potentially isolating effects of parasite-mediated sexual selection for bright colours (Chapters 3 and 4) could further contribute to the maintenance of reproductive isolation between *P. pundamilia* and *P. nyererei*.

Over the last decades, water transparency in Lake Victoria has been declining (Verschuren et al. 2002). Because many haplochromine cichlids rely on visual cues for mate recognition (Seehausen & Van Alphen 1998; Knight & Turner 2004), increased turbidity poses a threat to species diversity because it affects the rate of hybridisation between closely related species (Seehausen et al. 1997a). Also *P. pundamilia* and *P. nyererei* increasingly hybridise as water transparency decreases, and in extremely turbid water, single populations of slightly variable coloration are found (Seehausen 1997). Given that interspecific mate choice is affected by water transparency, the nature and strength of sexual selection on male coloration *within* species may be affected too. Indeed, across populations of *P. nyererei*, water transparency and the intensity of male coloration are correlated (Seehausen et al. 1997a). However, two different mechanisms may account for this relationship: i) female mating preferences and male colour may co-evolve in response to variation in water transparency, or ii) the strength of selection on male coloration by female mating preferences may be a function of signal transduction through the water, without mating preferences evolving themselves. I investigated these alternatives in **Chapter 6**. I studied the mate choice behaviour of a *P. nyererei* population from turbid water, and I used the same laboratory set-up as in Chap-

ter 2 to allow for comparison of the clear and the turbid water populations. I found that in the population from turbid water, female preference for male red coloration is significantly weaker than in the population from clear water. I also found that both the amount and the hue of male red coloration differ significantly between populations, consistent with adaptation to the different photic habitats of the two populations. These findings suggest that the observed correlation between male coloration and water transparency is not mediated by environmental variation alone. Rather, they indicate that female mating preferences have evolved in response to this variation. This is the first evidence for intraspecific preference-trait co-evolution in cichlid fish, confirming the earlier suggestion that the decrease in Lake Victoria water transparency affects both interspecific mate choice and intraspecific sexual selection (Seehausen et al. 1997a). This underlines the importance of measures to counteract the ongoing eutrophication of Lake Victoria.

Chapters 7-8: the colour morphs of *Neochromis omnicaeruleus*

By definition, speciation by sexual selection requires variation in mate preferences and preferred traits within species. *Neochromis omnicaeruleus* is a suitable model system to study such intraspecific variation, because it is one of the most polychromatic haplochromines known (Seehausen 1996). In addition to the blotch polymorphism which is the subject of Chapter 8, there is considerable colour variation among the individuals belonging to the presumably ancestral, not-blotched phenotype: male coloration ranges from sky-blue to yellow-red and females are grey-blue to yellow (see Figure 7.1 on page 113).

In **Chapter 7**, I present the first investigation of the blue/yellow polychromatism of *N. omnicaeruleus*. In a wild population at Makobe Island, I found that male colour is associated with size and sexual maturity: yellow males are smaller than blue males and tend to be sexually immature. In females, size and maturity do 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 occurrence and timing of this transition is influenced by both environmental and genetic effects. Importantly, some males mature and may start breeding while they are still yellow. Because haplochromine colour patterns mediate inter- and intraspecific mate choice (Seehausen & Van Alphen 1998; Seehausen et al. 1999b; Knight & Turner 2004; this thesis), the blue-yellow polychromatism of *N. omnicaeruleus* might serve as a target for diversifying sexual selection and provide a starting point for species divergence. Indeed, this polychromatism resembles the colour variation present among sister species of other haplochromine cichlids (Florin 1991; Seehausen et al. 1999c).

In addition to the blue-yellow phenotypes, two types of blotched morphs occur in both sexes of *N. omnicaeruleus*: orange-blotched and white-blotched (Fig-

ure 8.4 on page 128). Similar blotch colour polymorphisms are observed in several East African haplochromine cichlids, often associated with sex reversal genes. In *N. omnicaeruleus*, blotch-linked dominant female determiners can be compensated by autosomal male rescue genes, required to create blotched males. These associations and interactions between sex and colour genes generate selection favouring matings between matching genotypes that yield even offspring sex ratios. Therefore, blotch polymorphisms and sex determination genes may play an important role in the evolution of haplochromine species diversity (Lande et al. 2001). In *N. omnicaeruleus*, matings between blotched phenotypes and those plain phenotypes that do not possess rescue genes yield distorted offspring sex ratios. Most colour-sex combinations exert mating preferences that would avoid this, but blotched females do not exert any preferences (Seehausen et al. 1999b). Possibly, the spread of assortative mating preference genes in blotched females is precluded by the extremely low abundance of blotched males in the population studied: whereas about half of all females is blotched, 99% of the males has the plain phenotype. Since laboratory experiments on the genetics of the system suggested that blotched males should not be uncommon, and because the blotched colour pattern seems very conspicuous, Seehausen et al. (1999b) hypothesised that blotched fish may suffer increased predation from visual hunters. This idea is investigated in **Chapter 8**. In a predation experiment using wild kingfishers, I found that blotched fish indeed incur more predator attacks. Under water observations in the lake further suggested that sexual dimorphism in behaviour may account for an additional risk for males. A population census however showed that blotched males are rare already as juveniles. Yet, female phenotype frequencies were inconsistent with disassortative mating that could account for a lower production of blotched males. Therefore, to explain the scarcity of blotched males in nature there should be selection against blotched males early in life. Alternatively, the genetic architecture underlying the phenotypic distribution of colour and sex is more complex than the minimum model deduced from laboratory crosses. In fact, my results emphasise the need for extensive study of the underlying genetics.

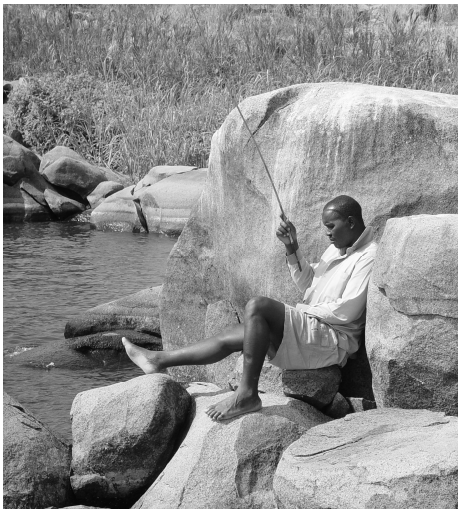
Whereas my study does not provide a conclusive explanation for the dynamics of the *Neochromis omnicaeruleus* blotch polymorphism, it does suggest that differential predation with regard to colour pattern and possibly sex, is an important selective force in the evolution and maintenance of mating preferences and colour gene expression. As such, my results indicate that natural selection by visual predators may hinder the establishment of conspicuous colour morphs in haplochromine cichlid fish. This may hold for other sexually selected colour patterns in haplochromine cichlids too. Thereby, predation may set a limit on the exaggeration of sexually selected traits and increase the potential for honest signalling.

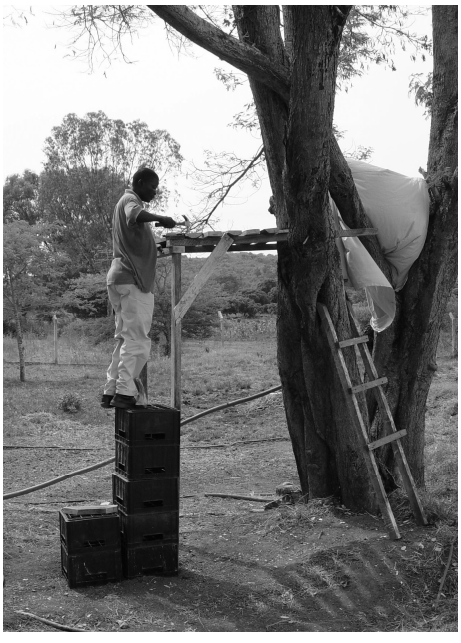
Chapter 9: Synthesis

In the final Chapter, I summarise the main findings of the work presented in this thesis. I conclude that the evolution of haplochromine colour patterns is subject to several simultaneous selection pressures. First, I found evidence for directional

sexual selection for male quality. Second, my work indicates that habitat heterogeneity, in terms of photic environment and parasite exposure, may promote population divergence in male nuptial coloration and female preferences. Consequently, I argue that sexual selection need not be 'Fisherian' in order to become divergent. Third, I found that predation pressure and water turbidity may constrain the evolution and persistence of conspicuously coloured morphs and species.

Together, these selection pressures and their interactions determine local species diversity. I discuss the implications of my findings for our understanding of haplochromine speciation, and I briefly indicate how my results may affect the direction of future work.





Chapter 2

'It is shown by various facts, given hereafter, and by the results fairly attributable to sexual selection, that the female, though comparatively passive, generally exerts some choice and accepts one male in preference to others. Or she may accept, as appearances would sometimes lead us to believe, not the male which is the most attractive to her, but the one which is the least distasteful.'

Darwin, 1871

Intraspecific sexual selection on a speciation trait, male coloration, in the Lake Victoria cichlid *Pundamilia nyererei*

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The haplochromine cichlids of Lake Victoria constitute a classic example of explosive speciation. Extensive intra- and interspecific variation in male nuptial coloration and female mating preferences, in the absence of postzygotic isolation between species, has inspired the hypothesis that sexual selection has been a driving force in the origin of this species flock. This scenario rests on the premise that the phenotypic traits that underlie behavioural reproductive isolation between sister species diverged under sexual selection within a species. We test this premise in a Lake Victoria cichlid, using laboratory experiments and field observations. We report that a male colour trait, that has previously been shown to be important for behavioural reproductive isolation between this species and a close relative, is under directional sexual selection by female mate choice within this species. This is consistent with the hypothesis that female choice has driven the divergence in male coloration between the two species. We also find that male territoriality is vital for male reproductive success and that multiple mating by females is common.

Introduction

The role of selection in the origin of new species is a relatively poorly understood problem in evolutionary biology and theoretical studies amply outnumber experimental ones (Kirkpatrick & Ravigne 2002). However, without empirical data on natural populations, it is impossible to judge how realistic model assumptions are.

The cichlid fish species flocks of the Great Lakes of East Africa, and the haplochromine radiations in Lakes Victoria and Malawi in particular, are classical examples of rapid speciation and adaptive radiation within the confines of single lakes. Lake Victoria is the youngest of the African Great Lakes (0.25-0.75 My; Fryer 1996) and sedimentological and paleoclimatic evidence indicates that it was completely dry as recently as 14.500 years ago (Johnson et al. 1996; Johnson et al. 2000). Although the genetic diversity contained in the flock must be far older (Nagl et al. 2000), most of the 500 or more endemic species must have diverged in an extremely short time. Several different models of speciation involving selection on mate choice traits have been proposed to explain this (Kocher 2004). Theoretically, mate choice can establish reproductive isolation very rapidly, since it directly influences mating behaviour and does not require postzygotic reinforcement through ecological selection against hybrids (Kirkpatrick & Ravigne 2002). Empirical support for sexual selection as a diversifying force has been demonstrated in birds (Barraclough et al. 1995; Uy & Borgia 2000; Irwin et al. 2001), lizards (Stuart-Fox & Owens 2003), parrotfishes (Streelman et al. 2002), insects (Arnqvist et al. 2000; Gray & Cade 2000), spiders (Masta & Maddison 2002), and snails (Schilthuizen 2003) but the evidence is not always unambiguous (e.g. Boake et al. 1997; Houde & Hankes 1997; Gage et al. 2002).

The appearance of and subsequent frequent changes in male nuptial coloration in East African cichlids is associated with the evolution of polygynous mating systems that would allow for stronger sexual selection (Seehausen et al. 1999a). Despite the morphological diversity that evolved in adaptive radiations of cichlids, closely related species of haplochromines typically have very similar morphologies and ecologies but differ strikingly in coloration (Albertson et al. 1999; Seehausen & Van Alphen 1999; Allender et al. 2003), with this variation affecting interspecific mate choice (Seehausen & Van Alphen 1998; Van Oppen et al. 1998). However, interspecific differences in characters subject to mate choice do not necessarily signify that sexual selection was the cause of their divergence. Additional support must come from studies that specifically test whether traits responsible for reproductive isolation *between* sister species evolve under sexual selection *within* species (Lande 1981; West-Eberhard 1983; Boake 2002). Such evidence is rare. One possible example is a North American butterfly in which intraspecific female mate choice using male wing melanisation is responsible for behavioural reproductive isolation between species (Wiernasz & Kingsolver 1992). In this paper we report experiments and field studies that constitute an explicit test of this prediction of the speciation-by-sexual-selection scenario in haplochromine cichlids.

Pundamilia pundamilia (Seehausen et al. 1998a) and *Pundamilia nyererei* (Witte-Maas & Witte 1985) are rock-dwelling haplochromine cichlids endemic to Lake Victoria. *P. nyererei* is sympatric with *P. pundamilia* in all of its range, the two are very closely related and exchange genes in some localities (Seehausen 1996; Taylor et al. in prep). They differ only slightly in anatomy and both species exhibit vertical melanin bars that are more pronounced in males than in females. However, whereas females of both species are cryptically coloured and can be distinguished only with difficulty, *P. pundamilia* males are metallic blue-grey and *P. nyererei* males are bright red dorsally and yellow laterally. This difference in male colour pattern is important in female mate choice: in laboratory experiments, females choose conspecific males when colours are visible, but not when they are masked (Seehausen & Van Alphen 1998).

Here we test within *P. nyererei* whether females select for brighter red males, using three approaches. First, we conduct mate choice experiments in the laboratory to test whether females prefer the more red one of two males, and whether the strength of preference increases with the magnitude of the difference in redness. Second, we investigate the importance of male coloration as a predictor of female responsiveness in nature, where female choice could also be influenced by extended phenotype traits and other sources of variation. Third, we test whether male coloration predicts male mating success in a mesocosm experiment.

Methods

Fish

We studied a *P. nyererei* population at Makobe Island in the western Speke Gulf (Tanzania; Seehausen & Bouton 1997), where the water is relatively clear (Secchi reading mean±se=221±15 cm in the study period). All experiments and observations were carried out in 2001. Mature *P. nyererei* males defend territories on rocky bottom at four to seven meters water depth. They attract females by vigorous courtship displays that resemble those of other haplochromine species (Seehausen & Van Alphen 1998). Courtship typically starts with Lateral Display, in which the male positions itself perpendicular to the female and spreads all fins. This is followed by Quiver, a high-frequency shaking movement of the body. Finally, the male leads the female to the centre of the territory in a quick swimming bout, often with exaggerated tail beats (Lead Swim). Mating occurs in rocky crevices, and immediately after spawning the mouthbrooding female leaves the territory. Females mouthbrood eggs and larvae for about three weeks, and guard the fry for an additional week after releasing them (Seehausen 1996).

Mate choice experiment in the laboratory

Two-way mate choice experiments were carried out following the method of Seehausen & Van Alphen (1998): each male was kept in one of two six-sided perspex enclosures (140 litres) on either side of an 1800-litres aquarium. In each enclo-

sure, shelter was provided by a brick on top of a PVC tube. These shelters were readily accepted by the males as the centres of their territories. In the middle of the main tank two large stones provided shelter for the test female and ensured that the males could not see each other. The water in the male enclosures was filtered internally, making chemical communication impossible. Air stones were present in both enclosures and in the main tank; water temperature was kept at 24-26°C.

We used 25 wild caught *P. nyererei*: 14 males, assembled into 7 male pairs of fixed composition, and 11 females. All males were photographed, measured and weighed. Pairs were assembled such that the redscore differences between males in a pair varied from 1 to 30% body coverage. Size differences between paired males were small and did not co-vary with colour differences (standard length (SL): high redscore: 95.3±5.7 mm, low redscore: 95.9±4.5 mm [paired t-test: t_6=-0.54, p=0.61]; weight: high redscore: 27.3±7.3 g, low redscore: 27.0±3.0 g [paired t-test: t_6=0.17, p=0.87]).

Males were released into their compartments the evening before trials. A maximum of three females was tested with one male pair in a day and at least two hours separated consecutive trials. Male pairs were exchanged after three successful trials or after two days. The position of the males (left or right side of the aquarium) was reversed each time the same pair was introduced. To start a trial, the test female was released in the middle of the tank. Behaviour was recorded with Observer 3.0 software (Noldus). Observation time started when the test female was within 30 cm of either one of the male compartments and stopped when she left this area. This distance was chosen because, in field observations, any fish within 30 cm of a *P. nyererei* territory elicits a behavioural response of the territory owner, indicating 30 cm as an interaction threshold distance. Trials were completed once 15 minutes of observation time had been collected.

Each female was tested once with every male pair, but in the analysis we included only those trials in which both males courted (performing Quiver or Lead Swim at least once) and the test female responded positively (approaching a male in response to his Lateral Display, Quiver or Lead Swim) to at least one of them (48 trials). For every male courtship display event, we recorded whether the female responded by approaching the male. The resulting proportion is our measure of female response to each male. The difference in female response to two males in a trial is our measure of female preference. We also recorded aggressive behaviour of the males (butting and biting attempts) and counted the total number of encounters of each male with the test female. Courtship intensity was defined as the mean number of courtship behaviours per encounter with the female and was calculated separately for Lateral Display, Quiver and Lead Swim.

Field observations

We studied 28 territorial males, located between four and seven meters water depth, individually recognisable by their coloration pattern and/or injuries or scars. Using SCUBA, we collected between 5 and 10 focal observations of 10 min-

utes duration each for each male, yielding a total of between 50 and 100 minutes observation time per male (total observation time: 1950 minutes). Observations were carried out between 09:00 and 13:00 hours and all observations for one male were completed within a period of 6 to 32 days. We recorded all interactions between these males and conspecific females. Female response was defined as the proportion of courted females responding positively (i.e. approaching the male) to male courtship. Aggressive behaviour towards females, including aggressive Lateral Display (interpreted as such when it was followed by Frontal Display or chasing or biting), was much more common in these observations than in the laboratory experiments. We therefore used the number of times a male quivered to a female as our measure of male courtship intensity. Quiver is only rarely displayed in antagonistic contexts, unless the antagonists are of similar size and the antagonism escalates into a fight. This would never be the case in interactions between territorial males and females in nature (SL of territorial males: 80.9±0.5 mm [n=28]; random sample of 45 adult females: 63.5±0.4 mm). We measured territory size (m^2) of each male; territory borders were determined by the aggressive behaviour that the owner showed towards other fish (con- and heterospecific). After completion of behavioural data collection, all males were caught either in gillnets or by hook and line, both using SCUBA. Immediately thereafter males were measured (SL; to the nearest 0,1 mm) and photographed for colour analysis.

Mesocosm experiment

We built three outdoor concrete ponds of about 3·2·1 m^3 (length·width·depth) each at the Mwanza station of the Tanzania Fisheries Research Institute (TAFIRI), on the eastern shore of the Mwanza Gulf. After collection from Makobe Island, fish were kept in groups of approximately 40 individuals in larger ponds for three weeks. After this acclimatisation period we finclipped all fish, photographed the males and assembled three groups of six adult males and 20 adult females that were released in each pond. Twenty PVC tubes (length 15 cm, diameter 10 cm) in each pond provided shelter. For a period of three weeks, starting one week after releasing the fish, we collected the eggs of every brooding female (10, 11 and 7 clutches for groups 1, 2 and 3). To do so, ponds were seined once a week. Average clutch size was 48±5 eggs; all eggs were preserved in absolute ethanol. We used the number of matings, i.e. the number of clutches completely or partially sired by a male, as a measure of male reproductive success.

DNA was extracted from ten offspring of each clutch using a Nucleon BACC2 kit according to manufacturer's protocol (Amersham). DNA was precipitated in ice-cold absolute ethanol, washed in 70% ethanol, air-dried for 20 minutes and dissolved in 30 µl sterilised water. After checking the extraction on a 0.8% agarose gel, DNA concentration was determined by spectrometry. We used four tetranucleotide microsatellite loci for paternity assignment (Ppun5, Ppun7, Ppun20, Ppun21; Taylor et al. 2002). For each sample, ~25 ng DNA, diluted in 1 µl MilliQ, was added to a PCR mixture containing 4.648 µl MilliQ, 0.2mM of each dNTP (Promega), 5 pmol of both forward- and reverse primer, 0.26 U Taq poly-

merase (Bioline), 2.0mM $MgCl_2$ and 1µl 10x Mg free reaction buffer (Bioline). PCR programs were: Ppun5: two step protocol: 3 min at 94 °C, followed by five cycles of 94 °C for 30s, 62 °C for 30s and 72 °C for 30s followed by thirty cycles of 94 °C for 30s, 60 °C for 30s and 72 °C for 30s, followed by one cycle of 10 min of 72 °C. Ppun7: standard protocol: 3 min at 94 °C, followed by thirty cycles of 94 °C for 30s, 57 °C for 30s and 72 °C for 30s, followed by one cycle of 10 min of 72 °C. Ppun20 and Ppun21: touch down protocol: 3 min at 94 °C, followed by eight cycles of 94 °C for 30s and 60 °C dropping one degree every cycle (down to 53 °C) for 30s and 72 °C for 30s, followed by 25 cycles of 94 °C for 30s, 53 °C for 30s and 72 °C for 30s, followed by one cycle of 10 min of 72 °C.

The result of the PCR reaction was checked on a 1.5% agarose gel together with a 100 bp ladder. Amplification products were resolved on an ABI 377 sequencer, processed in GeneScan 3.1 and scored in Genotyper 3.1 (Perkin-Elmer). Of 260 analysed offspring, 13 were discarded because of insufficient DNA. Exclusion probability was 99.8% and all males were excluded as sires except one for all remaining 247 larvae (in 28 clutches; 8.8±0.3 offspring per clutch).

Colour analysis

All males were photographed under standard conditions, placed in a perspex cuvette with water and gently squeezed between a grey PVC sheet and the front window. We used an SLR camera with 100 mm lens and one flash on either side. All pictures were digitised. In Photoshop 6.0 (Adobe Systems Inc.) we adjusted white balance with the aid of a white patch that was attached to the front side of the cuvette. We analysed the colours of the fish body, excluding fins and eyes, in SigmaScan Pro 4.0 (SPSS Inc.). To calculate colour scores, we defined criteria to delimit the body area covered by 'red' and 'yellow' by a combination of hue and saturation (red: hue=0-26 plus 232-255, saturation 40%-97%; yellow: hue=27-45, saturation 40-97%) and subsequently calculated the area of the fish body that matched these criteria, yielding a percentage of body coverage. We similarly defined criteria for 'blackness', to quantify the body coverage of the black vertical bars and ventral aspects of the body (black: intensity=0-75).

Data analysis

Comparisons of groups and bivariate relationships were analysed using paired t-tests and Pearson correlations for normally distributed data, and Wilcoxon signed ranks tests (for dependent samples), Mann-Whitney-U tests (for independent samples) and Spearman correlations for non-normally distributed data (SPSS 10.0; SPSS Inc.). Means of normally distributed data are given with standard errors.

For the mate choice experiments in the lab, we analysed the influences of male size and colour on female response on a continuous scale using models with a binomial distribution and a logit link function. We subsequently calculated probability curves for female preference using the same method. Reproductive success of males in the mesocosm experiment was expressed as a proportion of the

total number of matings in each group and therefore analysed using binomial models as well. Female response to male characteristics in the field were recorded as counts and analysed using models with a Poisson distribution and a log link function. All generalised linear models were calculated in R (Ihaka & Gentleman 1996; http://www.r-project.org). Stepwise removal of nonsignificant variables from saturated models yielded minimal adequate models; significance was determined by F-tests examining the change in deviance following removal of each variable. We checked for over- and underdispersion and adjusted test statistics (Venables & Ripley 2002).

Results

Mate choice experiment in the laboratory

Females responded stronger to the courtship of males with high redscores (paired t-tests comparing average response to both males over all trials per female: Lateral Display t_{10}=3.88, p=0.003; Quiver t_{10}=3.08, p=0.012; Lead Swim t_9=2.30, p=0.047; Figure 2.1a). To test the relative importance of male coloration and size

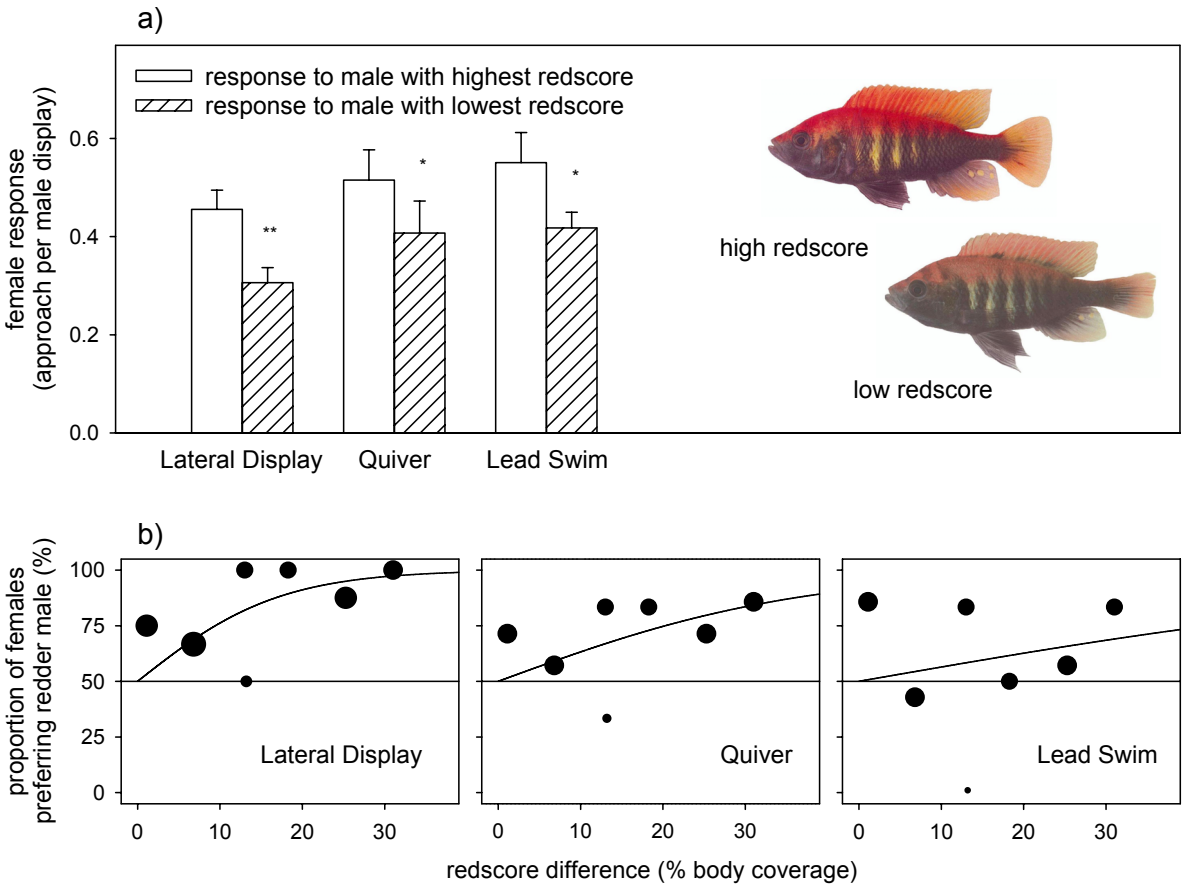

Figure 2.1 Mate choice experiment in the laboratory: a) mean and standard error of female response to males with high and low redscores. Data are pooled over all trials for each female. b) Probability curves for female preference depending on the difference in redscore between two males. For each male pair in the experiment, circles represent the proportion of females that preferred the redder male. Symbol sizes indicate sample sizes. Curves are forced through the origin.

we analysed female preference using generalised linear models with a binomial distribution, with logit-transformed female response as a dependent variable and male coloration (red, yellow and black), standard length and weight as independent variables. Only male redscore significantly influenced female response to male Lateral Display (estimate=4.25±0.79, $F_{1,47}$=35.13, p<0.0001) and Lead Swim (estimate=3.44±1.05, $F_{1,43}$=13.29, p<0.001). Female response to male Quiver was also best explained by male redscore (estimate=4.98±1.11, $F_{1,46}$=10.93, p<0.002) but significantly decreased with male blackscore (estimate=-1.51±0.65, $F_{1,46}$= 11.78, p<0.002). Besides the variation explained by covariates, there was no significant variation in response between females or between male pairs. Female responsiveness did not increase with the redness means of males in a pair (Spearman rank correlations: n=7, p>0.7), confirming that it was the difference in redscore between the two males rather than the absolute redscore of the preferred male that explained female response.

Males with high and low redscores did not differ in courtship behaviour, aggression or encounter rates with females (Wilcoxon Signed Ranks tests on medians of trials per male: n=7 male pairs, Z<1.19, p>0.24) and courtship intensity was not correlated with redscore (Spearman rank correlation of medians of trials per male: n=14 males; p>0.35 for each of the courtship behaviours). Female preferences were not correlated with behavioural differences between the males (n=48 trials; p>0.45).

In order to define the threshold above which females start discriminating between male redscores, we carried out a generalised linear model analysis with the difference in redscore as single explanatory variable and the number of females preferring either male as binomially distributed dependent variable. Figure 2.1b reports the predicted proportion of females preferring the reddest male in a pair, for any given difference in redscore between them (estimate [Lateral Display]=0.12±0.036, $F_{1,47}$=20.56, p=0.004; estimate [Quiver]=0.054±0.017, $F_{1,43}$= 13.12, p=0.011, estimate [Lead Swim]=0.026±0.022, $F_{1,39}$=1.42, p=0.29). The curves become less steep as the escalation of courtship behaviours proceeds, and the curve for Lead Swim is not significant. This could be a sample size effect since not all courtship bouts proceeded to higher escalation levels, resulting in decreasing numbers of trials that met calculation criteria in the sequence Lateral Display-Quiver-Lead Swim; and decreasing numbers of observations within trials in the same sequence. For Lateral Display, calculation criteria were met in all 48 trials (Lateral Display frequency 31.7±4.1 per male per trial), for Quiver in 44 trials (23.9±3.6) and for Lead Swim in 40 trials (18.8±3.1). The curves for Lateral Display and Quiver, both significant, indicate that females started discriminating as soon as there was a difference in redscore between the males.

Reproductive success in nature

Female response to male courtship was analysed in a generalised linear model including male territory size, water depth, male body size (standard length), redscore, blackscore, yellowscore and courtship intensity as independent variables

and female response to male courtship as dependent variable. Male redscore and courtship intensity significantly explained female response (estimate [redscore]= 0.046±0.016, $F_{1,26}$=10.25, p=0.004; estimate [courtship intensity]=0.415±0.142, $F_{1,25}$=7.66, p=0.011; Figure 2.2).

These effects were independent: redder males did not court more intensely (Pearson correlation, n=27 [one male did not court], r=0.16, p=0.44). The redscores of the males ranged from 0.3 to 28.7 % body coverage (15.4±1.5 %); a range similar to that among the males used in the laboratory experiment (3.3 to 34.3 %; 18.4±2.8 %).

There was a significant interaction between territory size and depth. Territories were clustered in two depth categories, with 11 males at 4.2±0.1 m depth and 17 males at 6.3±0.1 m depth. Territories in shallow water were larger than those in deeper water (4.14±0.81 m^2 and 2.03±0.36 m^2; t_{26}=2.70, p=0.012). Depth did not influence female choice, and redscore and courtship intensity did not correlate with depth or territory size. However, female choice for high redscore and courtship intensity also selected for males with territories that were large relative to others at the same depth (generalised linear model explaining female response by depth and territory size: estimate [territory size]= 0.137± 0.060, $F_{1,25}$=4.74, p=0.039).

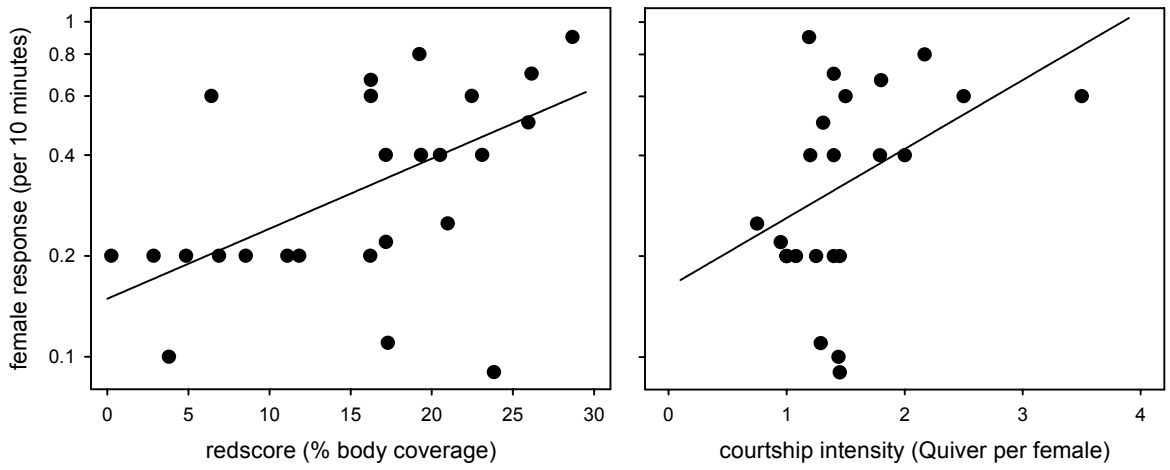

Figure 2.2 Field observations: the effects of male redscore (left panel) and male courtship intensity (right panel) on female response.

Mesocosm experiment

In two groups, three males (out of six) sired all genotyped offspring; in the third group, only two males (out of six) sired all offspring (Table 2.1). The majority of females had mated with more than one male: 19 of 28 broods had two or more fathers (68%; mean number of fathers per brood=1.8±0.1 across all broods). There was no correlation between the number of genotyped fry in a clutch and the number of fathers assigned (Spearman rank correlation: n=28 broods, r_s=0.16, p=0.41), indicating that we did not significantly underestimate the number of sires due to incomplete genotyping.

Table 2.1. Summary of the results of the mesocosm experiment.

		group		
		1	2	3
number of mated males		3	3	2
number of clutches		10	11	7
number of genotyped fry		89	92	69
number of clutches with				
one father	(% of clutches)	3 (30)	4 (36)	2 (29)
two fathers	(% of clutches)	4 (40)	6 (55)	5 (71)
three fathers	(% of clutches)	3 (30)	1 (9)	0 (0)
total multiple paternity	(% of clutches)	7 (70)	7 (64)	5 (71)

To determine which male characteristics influenced mating success, we calculated a generalised linear model with binomial distribution and logit link function using blackscore, redscore, yellowscore, male size (standard length) and 'group' as independent variables and the number of matings as dependent variable. The slopes of the relationships between male traits and the number of matings did not differ between the groups (all $F_{1,14}<0.86$, $p>0.37$). The minimal adequate model to describe mating success included blackscore only (estimate=2.19±0.76, $F_{1,16}=9.02$, $p=0.008$; Figure 2.3a, left panel). Including the effect of redscore did not improve the model ($F_{1,15}=0.32$, $p=0.58$). When blackscore was excluded from the model, there was a nearly significant trend for redder males to sire more offspring (estimate=1.76±0.91, $F_{1,16}=4.0$, $p=0.063$; Figure 2.3a, right panel). Redscore and blackscore were strongly correlated (Spearman rank correlation: $r_s=0.76$, $p=0.0004$).

The unmated males in the experiment had significantly lower blackscores than the mated males, and than the territorial males in our field study (Mann-Whitney U tests: compared to mated males in the experiment: $n_1=10$, $n_2=8$, $Z=2.67$, $p<0.01$; compared to field males $n_1=10$, $n_2=28$, $Z=3.35$, $p<0.001$; Figure 2.3b); whereas the mated males in the experiment did not differ from the territorial field males ($n_1=8$, $n_2=28$, $Z=0.50$, $p=0.64$). This suggests that the males with low blackscores in our experiments did not become territorial, which in turn implies that most unmated males possessed no territory. This makes the mesocosm data set different from our field and laboratory data sets which consisted entirely of territorial males. Because mesocosm males were photographed prior to the experiments, this also suggests that blackscore predicts future territoriality. We therefore repeated the analysis including only those males that had mated ($n=8$; 7 with high blackscores, one with low). In this subset, redscore emerged as the best, but again non-significant, predictor of mating success (estimate [redscore]=1.38±0.81, $F_{1,7}=3.05$, $p=0.13$; estimate [blackscore]=0.61±0.74, $F_{1,7}=0.70$, $p=0.44$). Redscore and blackscore were no longer correlated ($r_s=0.19$, $p=0.5$). Thus, this smaller data set resembled the territorial field males among which redscore and blackscore were not correlated either ($r_s=-0.15$, $p=0.44$).

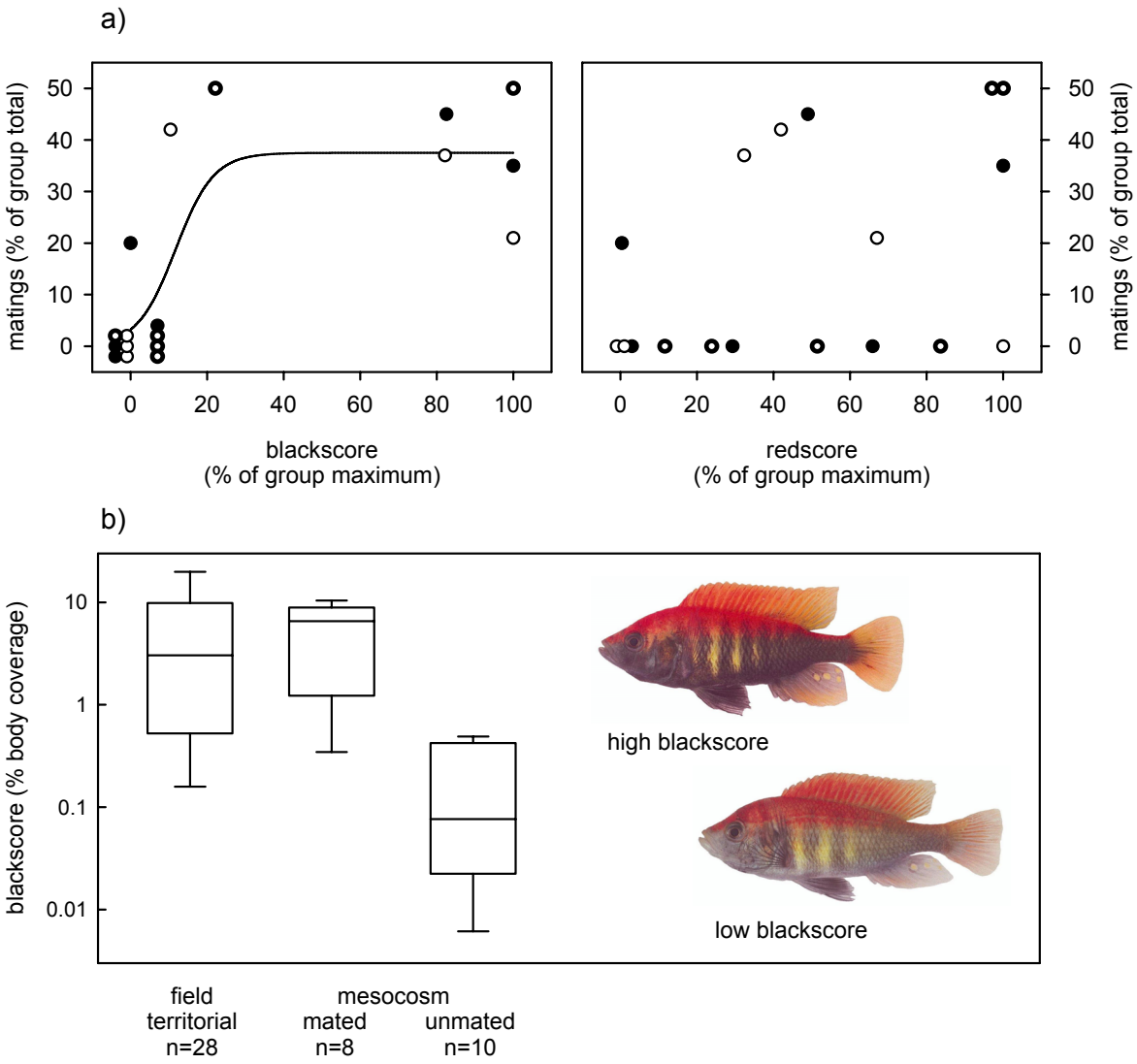

Figure 2.3 Mesocosm experiment: a) male blackscore (left panel) and redscore (right panel) and the number of matings in each group; filled circles: group 1, open circles: group 2, half-open circles: group 3. b) blackscores of territorial males in the field and of mated and unmated males in the mesocosm experiment. Boxes represent 75% confidence intervals intercepted by the median; error bars represent 95% confidence intervals.

Discussion

Two different mate choice experiments and a field study all suggest that the redness of males is the most important criterion for female mate choice among territorial males in *Pundamilia nyererei*. In a behavioural laboratory experiment, male redness predicted female response to courtship displays. Probability analysis indicated that the proportion of females preferring the redder one of two males increased with the magnitude of the difference between them. The predicted proportion of redder-preferring females exceeded 50% as soon as there was a difference between the males, suggesting that even a very small difference in redness might be enough to elicit a preference, and associated variation in mating

success on population level. However, our experiment included only two male pairs with redscore differences below 10% body coverage. We suspect that we would detect a female response threshold with more trials in this range. Male size and yellowscore did not influence female response; male blackscore significantly decreased female response to male Quiver but did not affect response to Lateral Display or Lead Swim.

The variation in redscores among males in the aquarium experiment resembled that among the field males. In nature however, females can use cues for mate choice decisions that were excluded in the experiment, such as chemical signals, territory size and water depth. Our field data reveal that even when these cues are available, male redness is the best predictor of female mate choice, with territory size a secondary factor. Male courtship intensity also predicted female response, possibly due to an interaction between male and female behaviour, with males displaying more as females stay in their territory longer (Collins 1994). In the laboratory experiment, the male enclosures did not allow females to actually follow a male into his spawning pit, making such behavioural interactions less influential. Results of the mesocosm mating experiment under semi-natural conditions are consistent with the field and laboratory results, but apparently highlight the importance of male territoriality for mating success. This may have implications for the potential of intrasexual selection (Seehausen & Schluter 2004; Van Doorn et al. 2004). The high incidence of multiple paternity that we observed resembles estimates for Lake Malawi haplochromines (Kellogg et al. 1995; Parker & Kornfield 1996; Knight & Turner 2004). In the field we frequently observed males courting females that were already mouthbrooding, illustrating the potential for multiple mating in nature.

Despite the prevalence of multiple paternity, territory ownership appeared to be crucial for male reproductive success. All matings in the mesocosms were monopolised by the blackest males in each group, except for one male with a near-zero blackscore that still mated with four females. Comparison to the blackscores of territorial field males suggests that blackscore is a correlate of territoriality. Even in aquaria, males (as well as females) switch on their black vertical bars when they become territorial. This is generally true in vertically barred haplochromines (Seehausen et al. 1999a) and observed in other cichlid species as well (Baerends & Baerends-Van Roon 1950; Barlow & Munsey 1976; Hulscher-Emeis 1992). Males used in the mesocosm experiment were photographed after an acclimatisation period of three weeks in the ponds but before the experiments were started. Although group composition in this period was different from that in the experiment, the relationship between blackscore and reproductive success suggests that territoriality during acclimatisation predicted territoriality during the experiment. If this suggestion is correct, it implies that alternative mating tactics are not very important in this species, which is consistent with observations in Lake Malawi haplochromines (Parker & Kornfield 1996) and with the theoretical prediction that alternative strategies primarily evolve when paternal investment is high (Taborsky 2001). Male *Pundamilia* do not contribute to broodcare, and pa-

ternal investment is confined to costs related to territoriality. We also never observed parasitic spawnings by non-territorial 'sneaker' males in the field, but spawning was observed only five times in 195 focal observations.

Unfortunately, the water in the ponds quickly became rather turbid and this made direct observations of male territoriality impossible. At the same time, high algal density hampers light transmission and selectively removes red light. This may provide an additional explanation for the finding that male reproductive success was not significantly related to redscore.

The correlation between redscore and blackscore among mesocosm males disappeared once unmated males were excluded, suggesting that red coloration is not fully expressed until males establish territories, a plausible strategy if the expression of coloration is costly. As a consequence, female choice would have been limited to only two or three males in each mesocosm group. With two or three territorial males in each pond, the territory density resembles the field situation in which territories typically occupy about three square metres.

We conclude that the conspicuous red coloration of male *P. nyererei* which is important in behavioural reproductive isolation between this species and its blue sister species (Seehausen & Van Alphen 1998), is subject to directional sexual selection by female mate choice within *P. nyererei*. This is consistent with a speciation scenario in which sexual selection through female choice has played an important role during the divergence of *P. nyererei* from *P. pundamilia*. Although we cannot currently prove it beyond doubt, all evidence suggests that *P. pundamilia* with metallic blue-grey males and females preferring them over red males (Seehausen & Van Alphen 1998), represents the ancestral condition (Seehausen et al. 1997a). *P. pundamilia* has a wider and more continuous geographical distribution, whereas that of *P. nyererei* is nested within the latter; and blue-grey is the dominant male colour pattern in the genus *Pundamilia* in general (Seehausen & Van Alphen 1999).

Colour polymorphisms in male nuptial coloration, involving blue-red or blue-yellow sister species and intraspecific morphs are common in haplochromine cichlids (Seehausen et al. 1999c). Here we have shown that male nuptial coloration is also under directional intersexual selection within a species. Similar results were recently obtained in a cichlid population of Lake Malawi (Pauers et al. 2004). The next challenge is to explain why and when such sexual selection becomes disruptive. This will require investigations into proximate (e.g. sensory) and ultimate causes of individual variation in mating preferences (Jennions & Petrie 1997).

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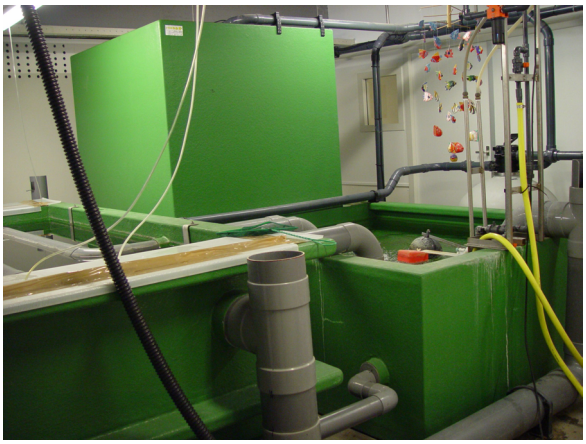
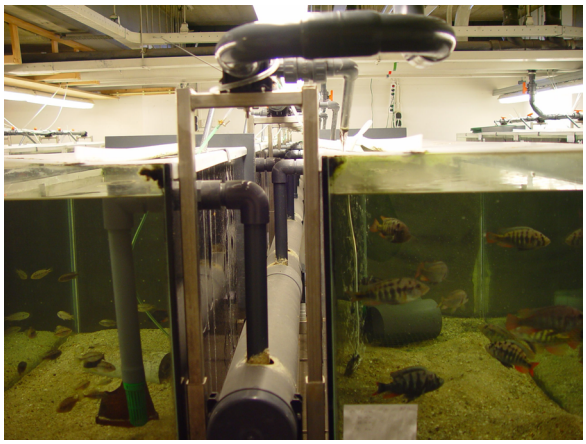

The aquarium system in which fish were kept and experiments carried out. The system was constructed in 2002 by Fleuren & Nooijen BV in the Gorlaeus Laboratory of Leiden University. The system harbours between one and two thousand fishes at any one time, in 183 aquaria. The water (18 thousand litres) is kept at a temperature of 24±1°C and is continuously filtered both mechanically and biologically. Photos by Kees Hofker; see also www.fleuren-nooijen.nl.

Chapter 3

'The development, however, of certain structures (…) has been carried to a wonderful extreme which, as far as the general conditions of life are concerned, must be slightly injurious to the male. From this fact we learn that the advantages which favored males derive from conquering other males in battle or courtship, and thus leaving a numerous progeny, are in the long run greater than those derived from rather more perfect adaptation to their conditions of life.'

Darwin, 1871

Fitness correlates of male coloration in a Lake Victoria cichlid fish: evidence for parasite-mediated sexual selection on a speciation trait

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(submitted)

Sexual selection by female choice has contributed to the rapid evolution of phenotypic diversity in the cichlid fish species flocks of East Africa. Yet, very little is known about the ecological mechanisms that drive the evolution of female mating preferences. We studied fitness correlates of male nuptial coloration in a member of a diverse Lake Victoria cichlid lineage, *Pundamilia nyererei*. In this species, male red coloration is subject to intraspecific sexual selection by female mate choice. Male nuptial coloration also underlies reproductive isolation between this species and the closely related sympatric species *P. pundamilia*. Here, we show that *P. nyererei* male coloration is carotenoid based, illustrating the potential for honest signalling of individual quality. In a wild population, we found that variation in male coloration was not associated with variation in a set of strongly intercorrelated indicators of male dominance: male size, territory size and territory location. Instead, the male characters that determine female choice, territory size and red coloration, may be independent predictors of male quality: males with bright red coloration and large territories had lower parasite infestation rates. As a result, female preferences tended to select against heavily parasitized males. Consistent with parasite-mediated sexual selection, males had higher and more variable parasite loads than females.

Introduction

The several hundred species of haplochromine cichlid fish endemic to Lakes Victoria and Malawi are textbook examples of explosive speciation (Fryer & Iles 1972; Schluter 2000; Kocher 2004). Female mate choice has played an important role in the phenotypic diversification of these species flocks: female preferences for male coloration exert sexual selection within species (Chapter 2; Pauers et al. 2004) and maintain reproductive isolation between sympatric incipient and sibling species (Seehausen et al. 1997a; Knight et al. 1998; Van Oppen et al. 1998; Seehausen 2000). Little is known however about the ecological mechanisms that determine the origin and evolution of these mating preferences: what do females gain by being choosy with regard to male coloration? Identifying the mechanisms by which female preferences evolve is fundamental for understanding mechanisms of rapid speciation.

Theoretical models that investigate the odds of speciation by sexual selection often rely on the assumption that that female preferences are selectively neutral (or entail a small cost) and do not enhance offspring survival (e.g. Lande 1981; Turner & Burrows 1995; Van Doorn et al. 2004; for reviews see Panhuis et al. 2001; Turelli et al. 2001). This assumption allows evolution of preference and trait in many directions and facilitates divergence, but it is opposed by numerous studies describing sexually selected traits that reliably indicate individual quality (for reviews, see Andersson 1994; Candolin 2003; Neff & Pitcher 2005). Female preferences for males that provide genetic quality or direct benefits are subject to natural selection and may not diverge so easily (Kirkpatrick & Nuismer 2004; but see Lorch et al. 2003; Edelaar et al. 2004; Reinhold 2004). Therefore, to understand the role of sexual selection in speciation we must identify the selection pressures acting on mating preferences.

We have previously demonstrated directional sexual selection on male red coloration in the Lake Victoria cichlid *Pundamilia nyererei* (Chapter 2). Male coloration is important also in interspecific female mate choice, which is the main source of reproductive isolation between *P. nyererei* and its close relative *Pundamilia pundamilia* (Seehausen & Van Alphen 1998). Laboratory breeding of *P. nyererei* indicates that both male coloration and female preferences are heritable (Seehausen et al. 1997a; Haesler & Seehausen 2005), making a coevolutionary process feasible. Here, we study the other extreme of the 'sexual selection continuum' (Kokko et al. 2002), where female selectivity may increase offspring health and survival. We investigate possible sources of selection on female mating preferences within *P. nyererei*. Lake Victoria haplochromine cichlids are female mouthbrooders and males do not contribute to brood care. Direct benefits are therefore unlikely to influence female choice, but bright colours may signal heritable quality through a variety of mechanisms (Andersson 1994). We ask whether male coloration is an indicator of male quality, and thereby a predictor of offspring viability. Specifically, we study the potential for parasite-mediated sexual selection (Hamilton & Zuk 1982) in a wild *P. nyererei* population.

Red, orange and yellow ornaments are often due to carotenoid deposition (e.g. in fish: guppies [Kodric-Brown 1989], sticklebacks [Brush & Reisman 1965] and cichlids [Evans & Norris 1996]). Besides their role in colour signals, carotenoids are attributed various beneficial physiological functions, particularly in immune defence (Olson & Owens 1998; Hill 1999). Animals obtain carotenoids from food (Goodwin 1984), but they are considered a scarce resource (Grether et al. 1999) and may constitute a trade-off between sexual ornamentation and survival (Folstad & Karter 1992; Lozano 1994). Carotenoid-based sexual ornaments thus fulfil the requirements of an honest signal of quality (Zahavi 1975) and several examples of this mechanism have been found, especially in birds (Saino et al. 1999; McGraw & Ardia 2003; Saks et al. 2003) and fish (Barber et al. 2001; Grether et al. 2004).

We test whether the red and yellow colours of male *P. nyererei* are carotenoid-based. Subsequently we investigate in wild territorial males whether yellow and red coloration and/or other visible characteristics predict parasite load, and whether the latter is related to variation in mating success measured in the wild. Because females may select high-quality mates by choosing males that are dominant in male-male competition (Cox & LeBoeuf 1977; Berglund et al. 1996), we also investigate the relationships between mating success, male coloration, parasite load and indicators of male dominance such as male size, territory size and territory location.

Methods

Behavioural observations and fish collection

We studied a *P. nyererei* population between December 2000 and February 2001 (subsequently referred to as '2001') and between November 2002 and March 2003 ('2003') at Makobe Island in the western Speke Gulf, Tanzania (Seehausen & Bouton 1997). The relatively clear water at this island allows underwater observations (Secchi reading mean±se=221±7 cm in the study period [84 measurements]). *P. nyererei* is mainly zooplanktivorous and also feeds on insect larvae and Nostoc (Bouton et al. 1997). Mature *P. nyererei* males defend territories on the rocky bottom at four to seven meters water depth and they attract females by vigorous courtship displays (Seehausen & Van Alphen 1998). When mating occurs, the female takes the fertilised eggs in her mouth and leaves the territory. Females mouthbrood their clutches for about three weeks and after first releasing them, guard the fry for another week (Seehausen 1996). Females and non-territorial males inhabit the same depth range and are typically seen in large feeding shoals with other zooplanktivores.

Observations on individually marked territorial males

Using SCUBA, we identified 28 territorial males in the year 2001, and collected between 5 and 10 observations of 10 minutes each for every male, yielding a total of 50 to 100 minutes observation time per male. We recognised males individually

by their coloration pattern and/or injuries or scars. Observations were carried out between 09:00 and 13:00 hours and all observations for any one male were completed within a period of 6 to 32 days. We recorded all interactions between these males and conspecific females. Female response was defined as the number of courted females responding positively (i.e. approaching the male) to male courtship. We used the number of male quivering bouts displayed to a female as a measure of male courtship intensity. We measured water depth at the territory (T depth; m) and territory size (T size; m^2); territorial borders were deduced from the positioning of the territory owner during aggressive behaviour towards other fish. We counted and measured (diameter, cm) all rocks in each territory and determined the number of crevices between rocks that were used by the territory owner. Territories were bimodally distributed over the sampled depth range with concentrations at depths of 4.21±0.06 m (n=11) and 6.26±0.06 m (n=17).

In the year 2003, we found *P. nyererei* territories in a continuous depth range from 4.3 to 6.5 meters in the same area of Makobe Island. Territory size and depth distribution did not differ between years (average territory size and depth: t<1.2, p>0.24; depth distribution: Chi^2=5.67, df=6, p>0.2). We identified 23 territorial males and measured territories of 14 of these males. We caught 8 males holding measured territories and 9 males holding territories that were not measured. Behavioural observations were not conducted.

In both years, territorial males were caught in gillnets or by hook and line, both using SCUBA. They were slowly brought to the surface in transparent plastic bags, which minimised the occurrence of stress-induced colour changes.

Observations on additional males and females

We collected an additional sample for parasite load analysis in 2001: 26 males and 19 females caught in gillnets (l·h: 30·1.5 m; stretched mesh sizes of 12.5, 16.5 and 18.5 mm) at approximately 6 meters water depth. Nets were set for a maximum of one hour to avoid colour loss in males. Female abundance at different depths was determined in 2003. We set 12.5 mm gillnets at 3.5, 4, 5 and 6 meters depth in the study area, and collected 104 females in 380 minutes. This sample emanated from experimental fishing for other species and we could not retain these fish. Therefore, these catches could only be used to determine the numbers of *P. nyererei* females at different depths, and fish were not dissected. To determine the depth distribution of females in relation to gonadal maturation stages we used a second sample, taken at 3.5, 4, 5, 6 and 6.5 meters depth (138 females).

Preservation

Immediately after capture, males were photographed for colour analysis. Fish were sacrificed on melting ice and measured (standard length: SL; to the nearest 0.1 mm). Fish were preserved in 4% formalin, the body cavity slit open ventrally to allow preservation of organs and internal parasites. After 1-4 weeks they were transferred to 30% ethanol, at least one week later to 60% and again at least one week later to the final solution of 70%. They were then weighed [W; to the nearest

0.1 g]) and condition factor (CF) was calculated as $CF=100\cdot(W/SL^3)$ (Sutton et al. 2000).

Colour analysis

For photography, males were placed in a perspex cuvette with water and gently squeezed against the front window of the cuvette with a grey PVC sheet. For all pictures, we adjusted white balance in PhotoShop 6.0 (Adobe Systems Inc.) using a white patch (Kodak colour card) attached to the front of the cuvette. We calculated colour scores in SigmaScan Pro 4.0 (SPSS Inc.). Following Chapter 2, we used criteria delimiting the body area (excluding fins and eyes) covered by red and yellow based on a combination of hue and saturation (red: hue=0-26 plus 232-255, saturation 40-97%; yellow: hue=27-45, saturation 40-97%) and subsequently calculated the area of the fish body that matched these criteria, This yielded a percentage of body coverage, subsequently referred to as 'redscore' and 'yellowscore'. We similarly defined criteria for blackness to quantify the body coverage of the black vertical bars and ventral aspects of the body (intensity=0-75; 'blackscore').

In 2001, we used an SLR camera and two flashes on either side and subsequently digitised pictures. In 2003, we used a digital camera without flash. To enable comparison of colour scores between years, we calculated colourscores of the Kodak colour card that was photographed with both the SLR and digital camera ($n=10$ pictures for each camera). This yielded a calibration factor of 1.16 for both red- and yellowscore. Blackscore was too different between methods to allow for calibration. When using calibrated colourscores, this is indicated in the text.

Determination of parasite load and sexual maturity

With a dissecting microscope, we examined the skin, fins, gills, abdominal cavity, gonads, liver and gastro-intestinal tract and counted all parasites. Parasite identification followed Paperna (1996). We report parasite counts for each parasite species separately. We calculated additional summary variables as estimates of overall parasite infestation rate: TPL (total parasite load) is the sum of all parasites infecting one fish; PS is the total number of parasite species infecting one fish. For territorial males, we calculated a third summary variable (median parasite load, MPL) that takes the differences in abundance between parasite species into account: for each species, we normalised the number of individuals infecting one fish with the median parasite load of that species in the sample of territorial males of either year, and summed these relative loads.

Sexually mature, gravid, females were those that were ready to spawn within a few days, i.e. their ovaries were full with eggs that had a diameter of at least 80% of the maximum observed. Sexually mature males had their testes swollen to more than 80% of the maximum observed.

Skin pigment analysis

One female and one male, adult F1 offspring of wild caught *P. nyererei* from Makobe, were sacrificed on ice. Of each fish, two skin samples of approximately 2 cm^2 were taken: one from the dorsum (red in males), the other from the side of the fish (yellow in males; both brownish in females). These were dried for a few minutes and then weighed (g) and measured (mm^2). Pigments were extracted in acetone for 24 hours. Absorption spectra of the acetone extracts, measured in the range of 220-600 nm in a spectrophotometer (Unicam UV1) showed that male skin samples significantly absorbed in the 420-470 nm range, whereas female skin samples did not. Acetone extracts of the male skin samples were evaporated overnight, re-dissolved in hexane and absorption spectra determined. Carotenoid content of these extracts was estimated from absorbance at λ_{max} using the absorption coefficient $A^{1\%}_{1cm}$=2500 for carotenoid mixtures (Britton et al. 1995). Besides carotenoids, orange and red colour patterns in fish can contain drosopterins (Fox & Vevers 1960; Hudon et al. 2003). To detect these, hexane extracts were dried and re-dissolved in 30% EtOH, acidified with HCl to pH 2, and tumbled for 24 hours at room temperature. For comparison, drosopterins were extracted from 50 *Drosophila melanogaster* heads using the same procedure. Extracts were analysed using spectrophotometry and HPLC (Waters 990 photodiode array) with an Allsphere ODS 2 column (5 µm) (15 cm·4.6 mm) (Alltech) and a mobile phase of 70:20:10 (volume %) acetonitril:CH2Cl2:methanol (flow rate 1 ml/min, pressure 500 psi).

Data analysis

Comparisons of groups and bivariate relationships were analysed using paired t-tests and Pearson correlations for normally distributed data, and Mann-Whitney-U tests, Wilcoxon signed ranks tests, Chi-square tests and Spearman correlations for non-normally distributed data (SPSS 10.0, SPSS Inc.). Means of normally distributed data are reported with standard errors. Multivariate relationships were analysed with generalised linear models (GLM) using R (Ihaka & Gentleman 1996; http://www.r-project.org). Models with counts as dependent variables used Poisson distributions and logarithmic link functions. The proportion of gravid females was analysed with a binomial model and a logit link function. All other models assumed normal distributions; distributions of residuals were consistent with this assumption. Stepwise removal of non-significant variables from saturated models yielded minimal adequate models; significance was determined by F-tests examining the change in deviance following removal of each variable. In Poisson models, test statistics were adjusted for over- and underdispersion (Venables & Ripley 2002).

To determine whether and how variation in parasite load was reflected in variation in characteristics of territorial males that could be assessed by females, we calculated minimal adequate GLMs with parasite load as dependent variable and the following male characteristics as independent variables: male redscore, yellowscore, blackscore, standard length, courtship intensity, and territory size and water depth. Because male redscore, courtship intensity and territory size de-

termine female choice (Chapter 2), we report GLM results for these traits separately. Courtship intensity was recorded in 2001 only. In all other models, we pooled the data from both years and included 'year' as a factor. Because territory size varied with water depth, depth was included as a covariate in models with territory size as independent variable. For all tests of relationships with parasites, we applied sequential Bonferroni corrections (Sokal & Rohlf 1995), using k= the number of parasite species analysed or k= the number of summary variables.

Results

Yellow and red skin pigments are carotenoid-based

The absorption spectrum of the hexane extract from the red skin sample showed a typical carotenoid pattern: a shoulder at 418 nm, one peak at 439 nm and the highest peak at 468 nm. Total carotenoid content was 0.58 mg/g and 0.24 $\mu g/mm^2$. The yellow sample showed a very similar absorption spectrum: one peak at 416 nm, the highest peak at 439 nm and a third peak at 467 nm. Carotenoid content was 0.16 mg/g and 0.085 $\mu g/mm^2$. Thus, the red skin sample contained approximately three times more carotenoid than the yellow skin sample. The EtOH extract of the yellow skin sample did not contain drosopterins. Both the red skin extract and the *Drosophila* extract showed a broad absorption peak at 476 nm, corresponding to the known absorption peak of drosopterins in acid solution (475 nm, Needham 1974). The retention times of this compound in the HPLC were identical for the fish and the *Drosophila* extracts (2.06 minutes). For the red fish skin sample, the contribution of drosopterins to the total absorption of visible light was small: peak absorbance amounted to only 3.5% of carotenoid peak absorbance.

Territorial males: male colour is not related to body size or territory size

Male coloration did not covary with male size: none of the colourscores were related to male size in either year (Pearson correlations, standard length: all $p>0.17$; weight: all $p>0.29$). Likewise, male coloration was not related to territory size (Pearson correlations, all $p>0.40$).

Both territory size and male size varied with water depth (Figure 3.1 and Table 3.1). Male territories were larger in shallow water in both years. In 2001, males in shallow water were larger and had higher condition factors than males in deeper water. In 2003, there were non-significant relationships in the same direction. In 2001, males in deeper water had higher yellowscores ($n_1=17$, $n_2=11$, $t=2.36$, $p=0.029$) but not redscores ($p=0.18$). In 2003, similar trends were present (red: $r=0.46$, $p=0.067$; yellow: $r=0.43$, $p=0.089$). Blackscores tended to decrease with depth (2001: $t=1.91$, $p=0.079$; 2003: $r=-0.24$, $p=0.35$). The relationships between male size, territory size and territory depth were not affected by male coloration (GLM; all $p>0.20$ in both years). Thus, males with high (or low) colour scores were not larger and did not hold larger territories in either year, but males in deep water were smaller and tended to have higher colourscores.

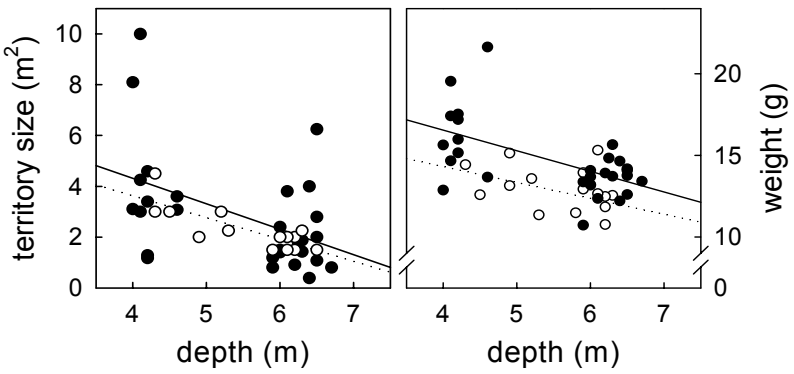

Figure 3.1 Territory size and male weight as functions of water depth in 2001 (black symbols, solid line) and 2003 (open symbols, dotted line).

Table 3.1 Means, standard errors and test results for the differences between deep and shallow territorial males in 2001 and 2003.

	2001				2003		
	shallow n=11	deep n=17	t	p	r	p	n
SL (mm)	82.6±0.8	79.8±0.6	2.95	0.0067	-0.18	0.48	17
weight (g)	16.5±0.8	13.6±0.3	3.58	0.0035	-0.39	0.12	17
condition (g/mm^3)	2.9±0.1	2.7±0.1	2.33	0.028	-0.28	0.28	17
territory size (m^2)	4.1±0.8	2.0±0.4	2.70	0.012	-0.80	<0.001	14

Deep and shallow habitats differed in several ways. First, although the number of rocks in a territory was identical (median=23 in both deep and shallow territories), rocks in shallow water were larger (rock diameter in shallow territories: 51±4.6 cm [n=11]; deep: 34±2.8 cm [n=17], t=3.35, p=0.0026) and provided more interstitial crevices (median and range 4 [1-5] versus 2 [1-4] per territory, MWU Z=-2.057, p=0.047; data from 2001). Second, the total abundance (catch per effort) of adult females increased significantly with increasing depth (GLM estimate=0.50±0.095, $F_{1,2}$=28.5, p=0.033). As the proportion of gravid females (i.e. ready to spawn) was larger in shallower water however (GLM estimate=-0.41±0.061, $F_{1,3}$=46.6, p=0.0064; data from 2003), the resulting expected number of gravid females that males would encounter did not differ between depth classes (Chi^2=3.32, df=3, p=0.34). In line with this, depth did not influence female choice (Chapter 2) and deep and shallow males did not differ in female response rate (n_1=17, n_2=11, MWU Z=-1.03, p=0.31), nor in the total number of interactions with females (Z=-0.59, p=0.58) or the number of aggressive interactions with females (Z=-1.16, p=0.26).

Territorial males caught in 2003 were significantly smaller than those caught in 2001 (n_1=28, n_2=17; standard length: 80.9±0.5 vs. 76.4±0.7 mm, t=5.1, p<0.001, weight: 13.7±0.4 vs. 12.7±0.4 g, t=3.12, p=0.0032) but there was no difference in condition factor (t=0.812, p=0.37). In 2001, male condition decreased with increasing redscore (n=28, r=-0.44, p=0.020). In 2003 this relation-

ship was absent (n=17; r=0.14, p=0.60). GLM analysis showed that this relationship differed significantly between years ($F_{1,42}$=4.98, p=0.031). Yellowscore and blackscore were not related to body condition in either year (Pearson correlations, all p>0.27). Males in 2003 had significantly higher scores for all colours (t>4.3, p<0.001). For blackscore, this difference may have been entirely due to the different cameras used. The differences in red-and yellowscore however remained significant after calibration (red: 2001: 17.8±1.5, 2003: 34.9±1.5, t=7.48, p<0.001; yellow: 2001: 1.7±0.4, 2003: 4.9±0.7, t=3.85, p=0.001).

Parasites

a) Parasite composition

We found seven species of macroscopic parasites: two endoparasites and five ectoparasites. In the skin and fins we found encysted metacercariae belonging to the trematode genus *Neascus* (Digenea). Three gill parasites were relatively common: two species of ectoparasitic copepods (*Lamproglena monodi* [Lernaeidae] and *Ergasilus lamellifer* [Ergasilidae]) and one monogenean (*Cichlidogyrus sp.* [Dactylogyridae]). Encapsulated larvae of an unidentified bivalve mollusc were present in the gills of a small number of fish. Larval nematodes (*Contracaecum sp.*) were commonly found in liver and abdominal cavity; trematodes (Digenea) were found in the intestines of a few fish in 2001 only.

b) Males have more parasites and larger variance in infestation rates than females

Males carried more parasites than females (Table 3.2 and 3.3a). However, male *P. nyererei* are larger than females and numbers of several parasites were related to fish size (GLM; results not shown). In a GLM with parasite loads as dependent variables and fish sex and size (standard length and weight) as independent vari-

Table 3.2. Parasite infestation rates of territorial males, unidentified males and unidentified females. % Denotes parasite prevalence: the proportion of infected individuals. TPL= total parasite load, PS= number of parasite species.

	territorial males 2001 (n=28)		territorial males 2003 (n=17)		unidentified males 2001 (n=27)		unidentified females 2001 (n=19)	
	%	median(range)	%	median(range)	%	median(range)	%	median(range)
Neascus sp.	89.3	2 (0-9)	100	5 (1-19)	46.2	0 (0-20)	52.6	1 (0-3)
L. monodi	89.3	5 (0-12)	100	7 (2-15)	84.6	2 (0-14)	84.2	3 (0-9)
E. lamellifer	78.6	1 (0-9)	64.7	2 (0-5)	53.8	1 (0-10)	36.8	0 (0-2)
mollusc larvae	14.3	0 (0-1)	47.1	0 (0-12)	7.7	0 (0-1)	10.5	0 (0-1)
Cichlidogyrus sp.	100	18.5 (4-52)	88.2	6 (0-12)	96.2	7 (0-41)	100	4 (1-11)
trematodes	10.7	0 (0-5)	0	0	11.5	0 (0-3)	5.3	0 (0-1)
nematodes	96.4	5.5 (0-136)	88.2	4 (0-20)	80.8	2.5 (0-112)	84.2	5 (0-56)
TPL		38 (19-166)		24 (11-52)		16 (4-124)		16 (6-70)
PS		5 (3-6)		5 (3-6)		4 (1-6)		4 (2-6)

Table 3.3. a) Differences in parasite load between males and females in 2001. b) Differences in parasite load between territorial males in 2001 and 2003. TPL= total parasite load, PS= number of parasite species. Test results that remain significant after sequential Bonferroni correction are printed in bold script.

	a) Males (55) vs. females (19) (2001)			b) Territorial males 2001 (28) vs. 2003 (17)		
	MWU Z	p	sign	MWU Z	p	sign
Neascus sp.	1.49	0.14	♂ > ♀	3.11	**0.0019**	2001<2003
L. monodi	1.77	0.077	♂ > ♀	2.71	**0.0067**	2001<2003
E. lamellifer	2.90	**0.0038**	♂ > ♀	0.91	0.37	
mollusc larvae	0.43	0.67		2.49	0.013	2001<2003
Cichlidogyrus sp.	5.24	**<0.001**	♂ > ♀	4.78	**<0.001**	2001>2003
trematodes	0.85	0.40		1.38	0.17	
nematodes	1.02	0.31		0.68	0.50	
TPL	2.42	**0.016**	♂ > ♀	-2.59	**0.0096**	2001>2003
PS	2.24	**0.025**	♂ > ♀	-0.47	0.64	

ables, males still carried significantly more *E. lamellifer* and *Cichlidogyrus* than females ($F_{1,72}$>5.7, p<0.020). Because infestation rates of some parasite species were higher in territorial males than in unidentified males (Table 3.2), we also analysed a subsample including only sexually mature fish (40 males, 10 females). This yielded similar results to the previous GLM analysis, except for a now significantly higher number of parasite species (PS) in males ($F_{1,48}$=9.28, p=0.0038). The variance in parasite infestation rates was also larger in males (Wilcoxon signed ranks test comparing parasite load variance for each parasite species between males and females: Z=-2.197, df=6, p=0.028; including only sexually mature fish: Z=-2.366, df=6, p=0.018).

In 2003, total parasite load of territorial males was significantly lower than in 2001, mainly due to lower infestation rates of *Cichlidogyrus* (Table 3.3b). We found significantly more *Neascus* and *L. monodi* in 2003. Parasite load was not related to body condition in either year (males and females: all F<3.97, p>0.07). Including colourscores in the models for males did not influence these results.

c) Redscore and territory size predict male parasite load

Of all parasite species, only the nematodes were significantly related to male characteristics that could be assessed by visiting females: high redscore and small size (SL) predicted low nematode load (F ≥7.78, p≤0.008; Table 3.4; Figure 3.2). Large fish (SL) tended to carry fewer *E. lamellifer* (p=0.019, critical p after Bonferroni correction=0.010) and mollusc larvae (p=0.022, critical p after Bonferroni correction=0.013) in their gills. There were non-significant negative relationships between territory size and *L. monodi* infection (p=0.084) and between redscore and *Cichlidogyrus* (p=0.082). Intestinal trematodes were not analysed separately because of their low incidence, but they were included in the summary variables.

Table 3.4 Male parasite load and characteristics that can be assessed by visiting females. TPL: total parasite load, MPL: median parasite load, PS: number of parasite species. Test results that remain significant after sequential Bonferroni correction are printed in bold script. a) Minimal adequate GLM for the relationships between male parasite load and male characteristics that can be assessed by visiting females. b-d) GLM results for the relationships between male parasite load and redscore, territory size and courtship intensity. Except for the analyses involving courtship intensity, data of 2001 and 2003 are pooled and a factor 'year' is included in the models.

	a) minimal adequate model				b) relation to redscore		
	variable	estimate±se	F	p	estimate±se	$F_{1,42}$	p
Neascus sp.	SL	-0.043±0.039	1.19	0.28	+0.002±0.017	0.009	0.93
L. monodi	T size	-0.024±0.014	3.17	0.084	-0.010±0.012	0.63	0.43
E. lamellifer	SL	-0.124±0.051	5.91	0.019	+0.012±0.021	0.35	0.56
mollusc larvae	SL	-0.244±0.104	5.68	0.022	-0.020±0.057	0.12	0.73
Cichlidogyrus sp.	red	-0.019±0.011	3.17	0.082	-0.019±0.011	3.17	0.082
nematodes	red	-0.094±0.027	10.90	**0.0020**	-0.081±0.030	7.78	**0.0079**
	SL	+0.194±0.065	8.77	**0.0051**			
TPL	red	-0.024±0.011	9.59	**0.0041**	-0.031±0.010	8.83	**0.0049**
	T size	-0.028±0.010	8.32	**0.0070**			
MPL	T size	-0.032±0.011	9.22	**0.0047**	-0.020±0.011	4.32	0.044
	red	-0.014±0.011	4.16	0.050			
PS	T size	-0.005±0.003	3.17	0.084	-0.006± 0.004	2.08	0.16

	c) relation to territory size			d) to courtship intensity (2001)		
	estimate±se	$F_{1,33}$	p	estimate±se	$F_{1,26}$	p
Neascus sp.	-0.017±0.020	0.78	0.38	-0.183±0.258	0.52	0.48
L. monodi	-0.024±0.014	3.17	0.084	-0.190±0.197	0.96	0.34
E. lamellifer	-0.037±0.023	2.96	0.094	-0.464±0.336	2.03	0.17
mollusc larvae	-0.006±0.074	0.007	0.93	+0.295±0.682	0.17	0.68
Cichlidogyrus sp.	-0.017± 0.010	2.74	0.11	-0.172±0.177	0.98	0.33
nematodes	-0.086±0.049	4.51	0.041	-0.518±0.685	0.61	0.44
TPL	-0.034±0.012	9.97	**0.0034**	-0.309±0.203	2.43	0.13
MPL	-0.036±0.012	11.06	**0.0022**	-0.400±0.175	5.55	0.026
PS	-0.005±0.003	3.17	0.084	-0.036±0.051	0.51	0.48

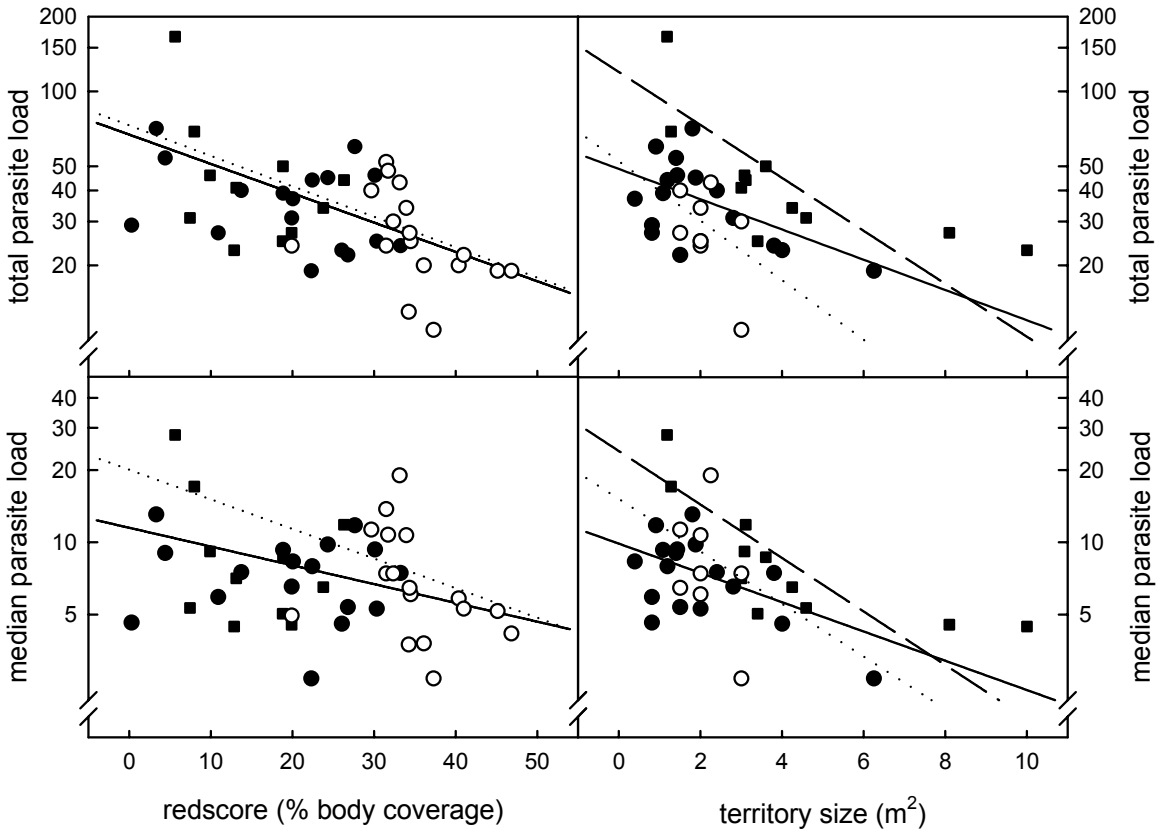

Figure 3.2. Male parasite load and characteristics that can be assessed by females. Total parasite load is the sum of all parasites infecting one fish; median parasite load is the sum of normalised parasite loads. Black symbols indicate territorial males of 2001 in deep (●) and in shallow (■) water, open symbols (○) indicate territorial males of 2003. Left panels: total and median parasite load in relation to redscore. Solid lines represent 2001 males; dotted lines represent 2003 males. For 2001, redscores are adjusted for photography method (i.e. multiplied by 1.16; see methods). The trend in the lower left panel is not significant (see Table 3.4b; p=0.044; critical p after Bonferroni correction =0.025). Right panels: total and median parasite load in relation to territory size. Solid lines represent males with territories in deep water (2001); dashed lines represent males with territories in shallow water (2001); dotted lines represent 2003 males.

Both male redscore and territory size were significantly negatively related to total parasite load ($F \geq 8.32$, $p \leq 0.0070$) and a large territory also predicted low median parasite load ($F_{1,33}$=11.06, p=0.0022). The number of parasite species infecting individual fish was not significantly related to any of the variables. Male courtship behaviour was not significantly related to parasite load ($p > 0.026$; critical p value after Bonferroni correction $p < 0.017$). Likewise, male yellowscore and blackscore were not related ($p > 0.10$) to infestation rates with any of the parasite species.

d) Females tend to select against heavily parasitized males

Previous work showed that female response rate was positively related to male redscore, courtship intensity and territory size (Chapter 2). There was a tendency for these preferences to yield selection against heavily parasitized males: there

were negative relationships ($p<0.05$) between female response and male parasite load for *Neascus* and *Cichlidogyrus* infection and for total parasite load (Table 3.5). After Bonferroni correction however, these relationships were not significant.

Table 3.5 Relationships between male parasite load and female response in 2001. TPL: total parasite load, MPL: median parasite load, PS: number of parasite species.

Male parasites and female choice (2001)		
	r_s	p
Neascus sp.	-0.43	0.023
L. monodi	-0.13	0.52
E. lamellifer	-0.01	0.97
mollusc larvae	0.20	0.31
Cichlidogyrus sp.	-0.39	0.039
trematodes	-0.19	0.32
nematodes	-0.26	0.18
TPL	-0.39	0.038
MPL	-0.32	0.10
PS	-0.14	0.48

Discussion

The red and yellow coloration of *Pundamilia nyererei* males is carotenoid based. Extracts from the red skin areas contained three times as much carotenoid as the yellow skin extracts. Drosopterins, present in the red skin extracts, contributed less than 4% to total absorbance. Because we used laboratory-bred individuals for the pigment analysis, we cannot rule out the possibility that the quality and quantity of the pigments encountered may differ from that in wild fish. However, the colours expressed by laboratory-bred *P. nyererei*, fed with a mixture of fresh shrimps and peas, are indistinguishable from those expressed by wildcaught individuals. Further, the differences in coloration that exist between different *P. nyererei* populations are maintained in laboratory-bred generations (Seehausen et al. 1997a).

We found no evidence that the redness of male coloration, which is the most important criterion for female mate choice (Chapter 2), is important in male dominance. It was not related to male body size or territory size. Territories in shallow water were larger, provided better shelter and were occupied by larger males. Since body size predicts fighting ability in male *Pundamilia* (Dijkstra et al. 2005), the observed distribution of males may result from competition among males with shallow water being the preferred habitat. At Makobe Island, both sexes of *P. nyererei* are most abundant around six meters depth (Seehausen & Bouton 1997; personal observation), suggesting that food conditions are not better in shallow water. Possibly, the larger rocks and greater number of crevices in shallow water provide better opportunities for predator avoidance. Moreover, territorial *P. nyererei* males compete for territories also with other haplochromine species. There is a general interspecific trend for larger species to inhabit shallower waters (Seehausen et al. 1998b). In our study, males in shallow water had frequent ag-

gressive interactions with *Neochromis omnicaeruleus* males (30% larger), whereas males in deep water had disputes predominantly with *Pundamilia* "pink anal" males, which are similar in size to *P. nyererei* (Seehausen 1996).

Most fish show indeterminate growth. Irrespective of the mechanism behind the observed distribution of territorial males, male size may thus be a reliable indicator of survival which in turn is expected to reflect genetic quality (Brooks & Kemp 2001). We did not find evidence that *P. nyererei* females preferred large males or males with territories in shallow water. Assuming that female choice is adaptive, this suggests that these traits do not reflect male genetic quality, or that other visible cues are better indicators.

Indeed, our data suggest that if female *P. nyererei* preference is aimed at discriminating against heavily parasitized males, they should pay attention to male red coloration and territory size. In two samples of territorial males that we took with an interval of two years, red coloration and territory size emerged as the best predictors of male parasite load. Most individual parasite species showed trends of being negatively correlated with both characters. This corresponds with the observation that male red coloration and territory size are important criteria for female mate choice in this species (Chapter 2). To our knowledge, this is only the second study of parasite-mediated sexual selection in cichlid fish (see also Taylor et al. 1998), and the first to implicate a carotenoid trade-off. Parasite load does not necessarily reflect the genetic quality of the immune system (Shykoff & Widmer 1996; Getty 2002): parasites may affect overall health and consequently weaken male competitive ability and colour expression. However, we did not find any relationship between parasite load and body condition. Assuming that feeding behaviour and physiological trade-offs have a genetic basis, it seems unlikely that the observed variation in parasite load is due to environmental effects alone. Therefore, our results may rather suggest that female choice for males that are bright red and possess large territories has evolved under selection for heritable fitness.

The result that redness rather than yellowness is related to both female choice and parasite load, is consistent with the idea that red is a more costly carotenoid display than yellow (Hill 1996), and is supported by the finding that the red areas of the male skin contain much higher carotenoid concentrations than the yellow areas.

The relationship between total parasite load and red coloration was similar in 2001 and 2003, but the territorial males in the 2003 sample were brighter red and carried fewer parasites, mainly due to lower infestation rates with *Cichlidogyrus* (Figure 3.2). We may speculate that female choice is driving the population temporarily towards lower parasite loads and associated increases in redscore, until another parasite species or another genotype of the same species comes to prevalence and causes a renewed increase in infestation rate. Perhaps more likely, infestation rates of *Cichlidogyrus* may have been lower in 2003 for environmental reasons, and subsequently have allowed for higher average redness. Territorial males were smaller in 2003 than in 2001, but this difference seems to be unrelated to the difference in redscore or parasite load: male size was not related to colour, and

parasites with higher infestation rates in larger fish (e.g. nematodes) were not less abundant in 2003.

Our observations that several parasite species occur in significantly larger numbers in males than in females, especially in territorial males (Table 3.2), and that infestation rates are significantly more variable in males, hint at male-specific trade-offs between immune defence and reproductive investment. These may be mediated by carotenoid-based male breeding coloration. Evidence for carotenoid limitation in males but not females has been found in guppies (Grether et al. 2004). However, the parasite species that was most strongly related to male coloration in our study, the nematode *Contracaecum*, did not differ in abundance between males and females. Conversely, the two species that were more abundant in males *(Ergasilus, Cichlidogyrus)* were not significantly related to male coloration. Perhaps the different parasite loads in males and females in *P. nyererei* are related to ecological differences between the sexes (Reimchen & Nosil 2001). Either way, the higher infestation rates of males make the production of carotenoid-based coloration particularly costly for males.

In contrast to selection for arbitrary traits, sexual selection for heritable fitness has been proposed to constrain rather than promote species divergence (Kirkpatrick & Nuismer 2004). This implies that our results present a challenge for the hypothesis that sexual selection has contributed to the divergence of *P. nyererei* and its close relative *P. pundamilia*. However, *P. nyererei* and *P. pundamilia* differ in diet and depth range (Seehausen 1996), which likely entails differences in parasite exposure. Consequently, directional sexual selection for extreme male phenotypes (Chapter 2; Pauers et al. 2004) may be accompanied by 'phenotype-matching' selection, due to preferences for locally adapted males. Theory suggests that the latter kind of selection has considerable potential to drive sympatric speciation (Arnegard & Kondrashov 2004; Edelaar et al. 2004; Reinhold 2004). Likewise, variation in female preferences could evolve by selection for MHC compatibility (Aeschlimann et al. 2003). Further work should evaluate the relative importance of these mechanisms for haplochromine speciation.

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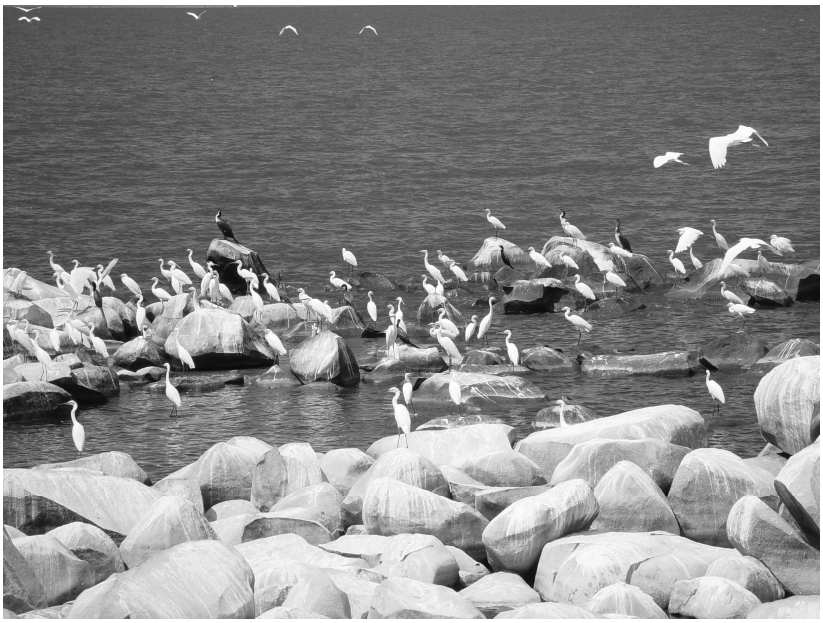
Chapter 4

'Under natural conditions it does not seem probable that beings so highly organized as fishes, and which live under such complex relations, should become brilliantly colored without suffering some evil or receiving some benefit from so great a change, and consequently without the intervention of natural selection.'

Darwin, 1871



Parasite-mediated sexual selection and species divergence in Lake Victoria cichlid fish

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(submitted)

We investigate the role of sexual selection for heritable quality in the evolution of haplochromine species diversity. These fish are classic examples of explosive speciation, possibly mediated by sexual selection: female preferences for male coloration exert sexual selection within species and maintain reproductive isolation between species. We study two anatomically similar sibling species that represent the major pattern of colour variation in haplochromines: male nuptial coloration is metallic blue in *Pundamilia pundamilia* and bright yellow and red in *Pundamilia nyererei*. Previous work indicated that the red coloration of *P. nyererei* is a carotenoid-dependent signal of parasite infestation rate. To understand the role of parasite-mediated sexual selection in the divergence of this species pair, we study the relationship between male nuptial coloration and parasite load in *P. pundamilia*. We find that *P. pundamilia* blue coloration is negatively correlated with parasite load. We also find that parasite infestation rates differ quantitatively between the species, in a way that is consistent with species differences in diet and microhabitat. We conclude that parasite-mediated sexual selection may have contributed to the divergence of *P. pundamilia* and *P. nyererei*, and may currently strengthen reproductive isolation between them.

Introduction

The hypothesis that sexual selection can drive speciation without geographical isolation has divided evolutionary biologists for decades, theoreticians and empiricists alike (Panhuis et al. 2001; Turelli et al. 2001). A few classic examples of explosive radiation suggest that it is possible, such as Hawaiian Drosophila's and East-African haplochromine cichlids (Dominey 1984). These radiations are characterised by the rapid evolution of hundreds of species, apparently without geographical barriers to gene flow. Further, the pronounced divergence in secondary sexual characters among incipient species that is accompanied by apparently only slight ecological differentiation, is consistent with strong divergent sexual selection (Albertson et al. 1999; Boake 2005).

Sustained coexistence of recently diverged species requires negative frequency-dependent selection. This can be achieved by intrasexual competition (Mikami et al. 2004; Seehausen & Schluter 2004; Dijkstra et al. 2005), but the potential and general importance of this phenomenon are questioned (Arnegard & Kondrashov 2004; Van Doorn et al. 2004). Alternatively, frequency-dependent selection arises through ecological divergence, a mechanism supported by a growing body of empirical evidence ('adaptive' or 'ecological' speciation; for reviews see Schluter 2001; Rundle & Nosil 2005).

We investigate how natural and sexual selection contribute to species divergence in East-African haplochromine cichlids. In Lakes Malawi and Victoria, hundreds of species have evolved very rapidly, apparently mediated by sexual selection: female preferences for male coloration exert sexual selection within species (Chapter 2; Pauers et al. 2004) and maintain reproductive isolation between species (Seehausen & Van Alphen 1998; Knight & Turner 2004). To elucidate the mechanisms driving haplochromine speciation, we study the selective pressures involved in the divergence of a sibling species pair from Lake Victoria. *Pundamilia pundamilia* and *Pundamilia nyererei* represent the dominant pattern of colour variation of the Lake Victoria haplochromine species flock (Seehausen et al. 1999c). *P. pundamilia* males are metallic grey-blue and *P. nyererei* males are yellow laterally and bright red dorsally. The two species are morphologically similar; the cryptically coloured females can be distinguished only with difficulty. Females of both species have assortative mating preferences and use male coloration as a choice criterion (Seehausen & Van Alphen 1998).

Within *P. nyererei*, females exert directional sexual selection on male red coloration (Chapter 2), possibly driven by 'good genes' selection: bright red males have lower macroparasite loads (Chapter 3). A similar mechanism may be present in *P. pundamilia*: if sexual selection on male coloration has contributed to the divergence of *P. pundamilia* and *P. nyererei*, we would expect blue-preferring females to receive some benefit from mating with bright blue males, just as red-preferring females do from mating with bright red males. In the present study, we therefore investigate the relationship between male coloration and parasite load in *P. pundamilia*.

P. pundamilia males are blue on the body and in the dorsal fin, but they also express red coloration, mainly in the caudal and anal fins. We quantify both aspects of male coloration and see whether they are related to parasite infestation rate in a sample of wild-caught adult males. We also analyse the carotenoid content in both components of *P. pundamilia* coloration, because carotenoids may mediate a trade-off between sexual signalling and parasite resistance (Lozano 1994). Previous work has shown that the red coloration of *P. nyererei* is based on carotenoids (Chapter 3). To determine whether similar trade-offs may exist in both species, we compare the colour pigments and parasite load of *P. pundamilia* with those previously documented for *P. nyererei*.

Methods

Fish collection and preservation

Using hook and line, we collected 26 males between December 2002 and March 2003 at Makobe Island (south-eastern Lake Victoria; western Speke Gulf). Immediately after capture, males were photographed for colour analysis. Fish were sacrificed on melting ice and measured (standard length (SL) to the nearest 0.1 mm and weight to the nearest 0.1 g). Fish were then preserved in 4% formalin, the body cavity slit open ventrally to allow preservation of organs and internal parasites. After 1-4 weeks they were transferred to 30% ethanol, at least one week later to 60% and again at least one week later to the final solution of 70%. We calculated condition factor (CF) as $CF=100\cdot(W/SL^3)$ (Sutton et al. 2000). Gonadal maturation was scored on a 5-point scale. Sexually mature males included those scored as 4 and 5 (i.e. testes swollen to more than 80% of the maximum observed). The amount of fatty tissue was scored on a 5-point scale, with 1 for individuals without any fat, and 5 for those with fat tissue completely covering the intestine and liver.

Colour analysis

For photography, males were placed in a perspex cuvette with water and gently squeezed against the front window of the cuvette with a grey PVC sheet. We used a digital camera (Sony DSC-F707) and adjusted white balance in PhotoShop 6.0 (Adobe Systems Inc.) using a white patch (Kodak colour card) attached to the front of the cuvette. We quantified the amount of red coloration in the dorsal, anal and caudal fins in SigmaScan Pro 4.0 (SPSS Inc.). We calculated the area of the fins that matched our redness criteria (a combination of hue [0-26 plus 232-255] and saturation [40-97%]; identical to those used for *P. nyererei* [Chapter 2]). This area was divided by the total fin area, yielding a percentage of fin coverage subsequently referred to as redscore. UV-reflectance of *P. pundamila* male nuptial coloration is negligible (Chapter 5). The blue coloration was quantified as follows (bluescore): we counted the number of blue-reflecting scales on the body and added this number to twice the number of blue-reflecting dorsal fin segments

(these segments occupy approximately twice the area of the scales). The number of scales was unrelated to male size (Pearson correlations, n=26, standard length: r=0.10, p=0.62, weight: r=0.10, p=0.63). To assess whether this measure corresponded to the brightness of the blue coloration as perceived by humans, we also scored blueness by eye on a scale from 0 (no blue at all) to 5 (bright blue in both dorsal fin and on the body). The two measures were highly correlated (Spearman correlation: r_s=0.65, p<0.001) and we continued the analysis with the more precise bluescore.

Determination of parasite load

With a dissecting microscope we examined the skin, gills, abdominal cavity, gonads, liver and gastro-intestinal tract and counted all parasites. Parasite identification followed Paperna (1996). We report parasite counts for each parasite species separately. We calculated additional summary variables as estimates of overall parasite infestation rate: TPL (total parasite load; the sum of all parasites infecting one fish), PS (the total number of parasite species infecting one fish), and MPL (median parasite load), which takes the differences in abundance between parasite species into account: for each species, we normalised the number of individuals infecting one fish with the median parasite load of that particular parasite species in the fish sample, and summed these relative loads.

Pigment analysis

We followed the same procedures as described previously for the closely related *P. nyererei* (Chapter 3). One adult male, laboratory F1 offspring of wild caught *P. pundamilia* from Makobe Island, was sacrificed on ice. Two pigment samples were taken: one from the skin of the side of the fish (grey-blue), the other from the fins (including soft part of dorsal, anal and caudal fins; bright red). These were dried for a few minutes and then weighed (g) and measured (mm^2). Pigments were extracted in acetone for 24 hours. These extracts were evaporated overnight and redissolved in hexane (8 ml for the red fin sample; 1 ml for the blue skin sample). Absorption spectra were determined in the range of 220-600 nm in a spectrophotometer (Unicam UV1). Carotenoid content was estimated from absorbance at λ_{max} using the absorption coefficient $A^{1\%}_{1cm}$=2500 for carotenoid mixtures (Britton et al. 1995). Besides carotenoids, red colour patterns in fish can contain drosopterins (Fox & Vevers 1960; Hudon et al. 2003). To detect these, the hexane-dissolved extracts were dried and re-dissolved in 30% EtOH, acidified with HCl to pH 2, and tumbled for 24 hours at room temperature. For comparison, drosopterins were extracted from 50 *Drosophila melanogaster* heads using the same procedure. Extracts were analysed using spectrophotometry and HPLC (Waters 990 photodiode array) with an Allsphere ODS 2 column (5 µm) (15 cm·4.6 mm) (Alltech) and a mobile phase of 70:20:10 (volume %) acetonitril:CH2Cl2:methanol (flow rate 1 ml/min, pressure 500 psi).

Species comparison

We compare the parasite infestation rates and pigment composition of *P. pundamilia* to that of a sample of territorial *P. nyererei* males that was collected at Makobe Island in the same period (Chapter 3). For this comparison, we include only sexually mature males.

Data analysis

Comparisons of groups and bivariate relationships were analysed using paired t-tests and Pearson correlations for normally distributed data, and Mann-Whitney-U tests and Spearman correlations for non-normally distributed data (SPSS 10.0; SPSS Inc.). Means of normally distributed data are reported with standard errors. We investigate the relationships between male parasite load and male visible characteristics (standard length, redscore, bluescore) for the subsample of sexually mature males, because these males are the ones available for female mate choice. We used generalised linear models (GLM) with Poisson distributions and logarithmic link functions, in R software (Ihaka & Gentleman 1996; http://www.r-project.org). Stepwise removal of non-significant variables from saturated models yielded minimal adequate models; significance was determined by F-tests examining the change in deviance following removal of each variable. Test statistics were adjusted for over- and underdispersion (Venables & Ripley 2002).

Results

Pigment analysis

The absorption spectrum of the hexane extract from the red fin sample showed a shoulder at 418 nm, one peak at 440 nm and the highest peak at 468 nm. This is a typical carotenoid pattern and it is practically identical to the spectrum described previously for the red pigment in *P. nyererei* (Chapter 3). Total carotenoid content in fins of *P. pundamilia* was 0.51 mg/g and 0.16 μg/mm^2 (*P. nyererei* red skin: 0.58 mg/g and 0.24 μg/mm^2). The blue skin sample showed a very similar absorption spectrum: one peak at 416 nm, the highest peak at 439 nm and a third peak at 468 nm. However, the carotenoid content was about 30× lower than in the red fin sample: 0.014 mg/g and 0.006 μg/mm^2. Given the areas on the fish body and fins that are covered with red coloration in both species, and assuming that the *P. nyererei* fins contain similar amounts of carotenoids as found in *P. pundamilia*, the total amount of carotenoid deposited in visible red coloration in *P. pundamilia* is approximately 10% of that in *P. nyererei*.

The EtOH extract of the blue skin sample did not contain any detectable amount of drosopterins. The red fin extract, like the *P. nyererei* red skin extract and the *Drosophila* extract (Chapter 3), showed a broad absorption peak at 476 nm, corresponding to the known absorption peak of drosopterins in acid solution (475 nm, Needham 1974). The retention times of this compound in the HPLC were identical for the fish and the *Drosophila* extracts (2.06 minutes). For the red fin sample of *P. pundamilia*, the contribution of drosopterins to the total absorp-

tion of visible light was small: peak absorbance amounted to only 4.4% of carotenoid peak absorbance (*P. nyererei*: 3.5%, Chapter 3).

Parasites

We found the macroparasite fauna to be composed of the same six parasite species as in *P. nyererei*: one endoparasite and five ectoparasites (Chapter 3). In the skin we found encysted metacercariae belonging to the trematod genus *Neascus* (Digenea). In the gills, we found two species of ectoparasitic copepods (*Lamproglena monodi* [Lernaeidae] and *Ergasilus lamellifer* [Ergasilidae]) and one monogenean (*Cichlidogyrus sp.* [Dactylogyridae]). Encapsulated larvae of an unidentified bivalve mollusc were present in the gills of a small number of fish. Larval nematodes (*Contracaecum sp.*) were commonly found in the liver and abdominal cavity.

Species comparison

P. pundamilia males had significantly more nematodes than *P. nyererei*, but *P. nyererei* carried significantly more copepods of both species, *L. monodi* and *E. lamellifer* (Tables 4.1 and 4.2). In *P. pundamilia*, total parasite load (TPL) was largely determined by nematode load and as a result significantly higher than in *P. nyererei*. The number of parasite species per individual fish (PS) was significantly higher in *P. nyererei*. Since parasite load may be limited by body size and *P. pundamilia* males were larger than *P. nyererei* males (weight [g]: n_1=14, n_2=17, mean±se 30.10±2.25 vs. 12.70±0.39, t=7.62, p<0.001), we also tested for species differences in parasite load divided by body weight (Table 4.2). Per gram bodyweight, *P. nyererei* had significantly higher loads of *Neascus*, *L. monodi* and *E. lamellifer* and a significantly higher number of parasite species (PS). *P. pundamilia* had higher numbers of *Contracaecum* per gram bodyweight than *P. nyererei*, but TPL per bodyweight did not differ significantly between the species.

Table 4.1 Descriptive statistics for parasite load in sexually mature males of *P. pundamilia* and *P. nyererei*. % denotes prevalence: the proportion of infected individuals.

	P. pundamilia (14 males)			*P. nyererei* (17 males)		
	%	mean±s.e.	median (range)	%	mean±s.e.	median (range)
Neascus sp.	100	6.64±0.78	6.5 (3-13)	100	5.76±1.05	5 (1-19)
L. monodi	50	2.0±0.73	0.5 (0-9)	100	7.71±0.89	7 (2-15)
E. lamellifer	14.3	0.14±0.097	0 (0-1)	64.7	1.76±0.41	2 (0-5)
mollusc	28.6	0.29±0.13	0 (0-1)	47.1	1.18±0.69	0 (0-12)
Cichlidogyrus sp.	92.9	8.36±1.27	8.5 (0-16)	88.2	5.47±0.75	6 (0-12)
nematodes	100	39.9±7.9	34 (1-94)	88.2	5.82±1.35	4 (0-20)
TPL		57.36±7.05	51 (25-110)		27.71±2.89	24 (11-52)
PS		3.86±0.25	4 (3-6)		4.88±0.27	5 (3-6)

Table 4.2 Mann-Whitney-U test results for the difference in parasite load between sexually mature males of *P. pundamilia* and *P. nyererei*. The direction of the difference is indicated for results with $p<0.10$; p>n indicates higher parasite load in *P. pundamilia*; n>p indicates higher parasite load in *P. nyererei*.

	raw data			controlled for body weight		
	Z	*p*		*Z*	*p*	
Neascus sp.	1.25	0.21		2.38	**0.017**	n>p
L. monodi	3.95	**<0.001**	n>p	4.67	**<0.001**	n>p
E. lamellifer	3.06	**0.002**	n>p	3.14	**0.002**	n>p
mollusc	1.20	0.23		1.72	0.086	n>p
Cichlidogyrus sp.	1.90	0.058	p>n	1.75	0.081	n>p
nematodes	3.90	**<0.001**	p>n	2.38	**0.017**	p>n
TPL	3.61	**<0.001**	p>n	0.87	0.38	
PS	2.47	**0.013**	n>p	4.45	**<0.001**	n>p

Parasite load in P. pundamilia

Larger *P. pundamilia* males had higher gonadal maturity scores (Spearman rank correlation, $n=26$: $r_s=0.82$, $p<0.001$) and higher bluescores ($r_s=0.54$, $p=0.004$). Redscore did not increase with male size ($r_s=-0.03$, $p=0.89$). Bluescore was not related to redscore ($r_s=0.14$, $p=0.51$). Some parasites increased in number with fish size (SL; *Cichlidogyrus*: $r_s=0.43$, $p=0.029$; *Neascus*: $r_s=0.35$, $p=0.077$); others were not related to male size (mollusc, *L. monodi*, *E. lamellifer*, nematodes: $r_s<0.3$, $p>0.13$).

Among the sexually mature males ($n=14$; Table 4.3 and Figure 4.1), large males had lower numbers of *E. lamellifer* in their gills and tended to carry fewer nematodes. As a result, they tended to have lower total parasite load (TPL), but not median parasite load (MPL) nor number of parasite species (PS). Males with high body bluescore had significantly lower *Neascus* load and tended to have a

Table 4.3 Parasite load and male size and colour in sexually mature *P. pundamilia* males ($n=14$).

	standard length			redscore			bluescore		
	$F_{1,12}$	*p*	effect	$F_{1,12}$	*p*	effect	$F_{1,12}$	*p*	effect
Neascus sp.	0.10	0.76	-	0.02	0.90	+	8.68	**0.012**	-
L.monodi	0.32	0.58	+	0.24	0.63	-	1.71	0.22	-
E.lamellifer	11.23	**0.006**	-	0.24	0.63	-	3.96	0.070	-
mollusc	2.17	0.17	-	0.11	0.75	+	3.06	0.11	-
Cichlidogyrus sp.	0.26	0.62	+	0.02	0.89	+	0.86	0.37	+
nematodes	3.81	0.075	-	3.32	0.094	+	0.47	0.51	+
TPL	4.34	0.059	-	4.28	0.061	+	0.27	0.61	+
MPL	0.84	0.38	-	0.12	0.73	+	4.94	**0.046**	-
PS	1.35	0.28	-	0.01	0.94	+	6.05	**0.03**	-

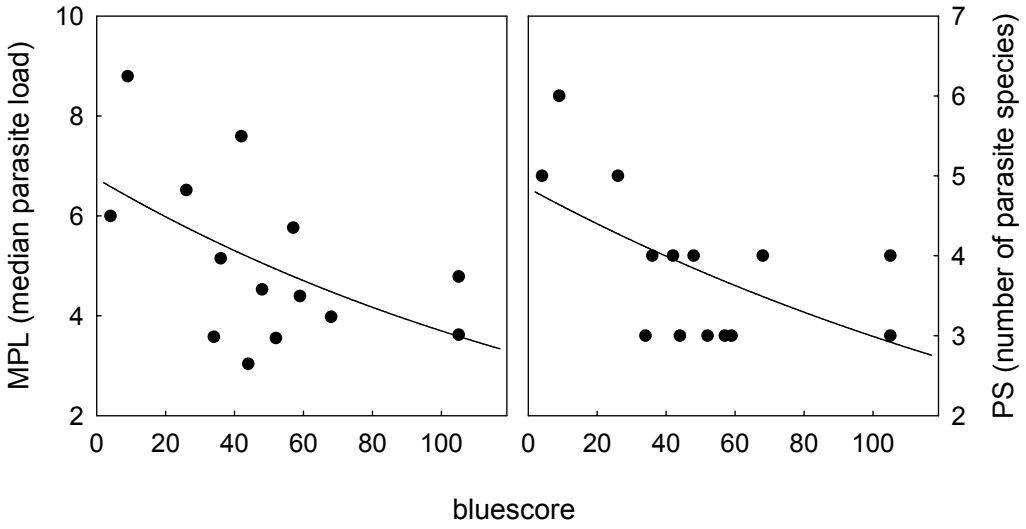

Figure 4.1 Bluescore and parasite load in sexually mature *P. pundamilia* males (n=14).

lower *E. lamellifer* load. Bluescore was the best, significant, predictor of low MPL and PS; standard length and redscore were not related to MPL or PS (p>0.28). Non-significant trends indicated that high fin redscores may predict high nematode loads and high TPL.

None of the parasites or summary variables was significantly related to body condition or fat content (Spearman rank correlations: $-0.35<r_s<0.35$, p>0.2). Redscore was positively related to body condition (r_s=0.53, p=0.049) but not fat content (r_s=0.05, p=0.86); bluescore was not related to body condition (r_s=0.03, p=0.93) but there was a tendency for a positive relationship with fat content (r_s=0.51, p=0.061).

Discussion

Pundamilia pundamilia and *P. nyererei* are two very closely related species of Lake Victoria cichlids, that form reproductively isolated and ecologically differentiated sympatric sister species in some localities, but interbreeding colour morphs or incipient species in other localities. We found the same species of macroparasites in both species (except for intestinal trematodes, that occurred in a few *P. nyererei* individuals [Chapter 3] but were absent in *P. pundamilia*). In both species, parasite infestation rates were below pathological levels (Paperna 1996). This is consistent with the observation that parasite load was not negatively related to body condition or fat content in either species.

The observation that parasite infestation rates differed quantitatively between *P. pundamilia* and *P. nyererei* indicates that the two species differ in their exposure to different parasite species, and/or in aspects of their immune defence. These are likely to be related to differences in habitat and diet: *P. pundamilia* feeds primarily on benthic insect larvae (Seehausen et al. 1998a) whereas *P. nyererei*

feeds primarily on zooplankton (Witte-Maas & Witte 1985). Both species inhabit rocky shores, but in the community that we studied *P. pundamilia* is most abundant in crevices between 0.5 and 1.5 meters depth and *P. nyererei* is most abundant outside crevices between 4 and 7 meters depth. The higher *Contracaecum* infestation rate in *P. pundamilia* is probably related to its shallow habitat: *Contracaecum* larvae depend on piscivorous birds as final hosts, in which they mature and produce eggs. These eggs wash into the water with bird excrements and infect their first intermediate host, small crustaceans, that are subsequently eaten by fish (Paperna 1996). At Makobe Island, egrets and cormorants aggregate in large numbers on the shoreline, covering the rocks with guano. As a result, the abundance of infectious *Contracaecum* stages is likely to decrease with depth and distance to the shore. In contrast, parasitic copepod loads (*E. lamellifer* and *L. monodi*) were higher in *P. nyererei*. These parasites reach their host directly via the water flow through the gills. Due to its zooplanktivorous, limnetic feeding style, *P. nyererei* may experience increased exposure to these free-living, pelagic copepods than does the more benthic-feeding *P. pundamilia* (Knudsen et al. 2004).

In *P. nyererei* males, low parasite loads are associated with high body redscores (Chapter 3). Although the red coloration of *P. pundamilia* fins seems to be chemically similar to that in *P. nyererei*, we did not find significant associations with parasite load. We found a non-significant trend for high redscores to predict high nematode load in *P. pundamilia*, whereas in *P. nyererei* there was a significant negative relationship between redscore and nematode load (Chapter 3). The relationships between body condition and redscore in the two species were inversed too: significantly negative in *P. nyererei*, but significantly positive in *P. pundamilia*. Thus, the information conveyed by carotenoid-dependent colour signals appears to differ between the species. *P. nyererei* males may be more carotenoid-limited than *P. pundamilia*, since they allocate more carotenoid to red coloration. Quantitative comparison however requires analysis of the carotenoid content in the diet of both species.

In *P. pundamilia*, high bluescores rather than redscores predicted low parasite loads: there was a negative relationship between bluescore and infection with *Neascus*, and there were trends ($p<0.10$) for negative relationships between bluescore and mollusc larvae and *E. lamellifer*. There were significant negative relationships between bluescore and median parasite load (MPL) and the number of parasite species (PS). Blue coloration is not based on pigments, but produced by microstructures that scatter long wavelengths and reflect short wavelengths. In contrast to carotenoid-based sexual ornaments, relatively little work has been done on the signal value of structural colours. There is evidence for sexually selected structural coloration in birds (Sheldon et al. 1999; McGraw et al. 2002; Doucet & Montgomerie 2003; Siefferman & Hill 2003) and fish (Kodric-Brown & Johnson 2002; Cummings et al. 2003; Boulcott et al. 2005), but the underlying physiological mechanisms are not resolved.

Females of *P. pundamilia* and *P. nyererei* use male nuptial coloration in interspecific mate choice (Seehausen & Van Alphen 1998) and in *P. nyererei*, male red

coloration is subject to intraspecific directional sexual selection by female choice (Chapter 2). We do not know whether female *P. pundamilia* similarly select for bright blue coloration among conspecific males, but the variation in male parasite load indicates that such mate choice could be adaptive. Our results therefore suggest that parasite-mediated sexual selection within each species could cause divergent selection between the species on male coloration and parasite resistance. Hence, divergent sexual selection may not be inconsistent with 'good genes' models of sexual selection.

During the divergence of *P. pundamilia* and *P. nyererei*, local adaptation to a heterogenous environment is likely to have played an important role. First, in the relatively turbid waters of Lake Victoria, the spectral environment changes rapidly with water depth, affecting selection on the visual system and male nuptial coloration (Chapter 5; Carleton et al. 2005). Second, depth differentiation coincides with differentiation in parasite exposure, through diet and through abundances of intermediate hosts or infective stages of parasites. Parasite-mediated sexual selection on male nuptial coloration may have contributed to the divergence of *P. pundamilia* and *P. nyererei*, and/or may currently strengthen reproductive isolation between them. Since blue vs. red and blue vs. yellow polymorphisms or sister species are common among haplochromine cichlids (Seehausen et al. 1999c), and given the evidence for parasite-mediated sexual selection also in other cichlid species (Taylor et al. 1998), this mechanism may not be unique to the species pair we studied.

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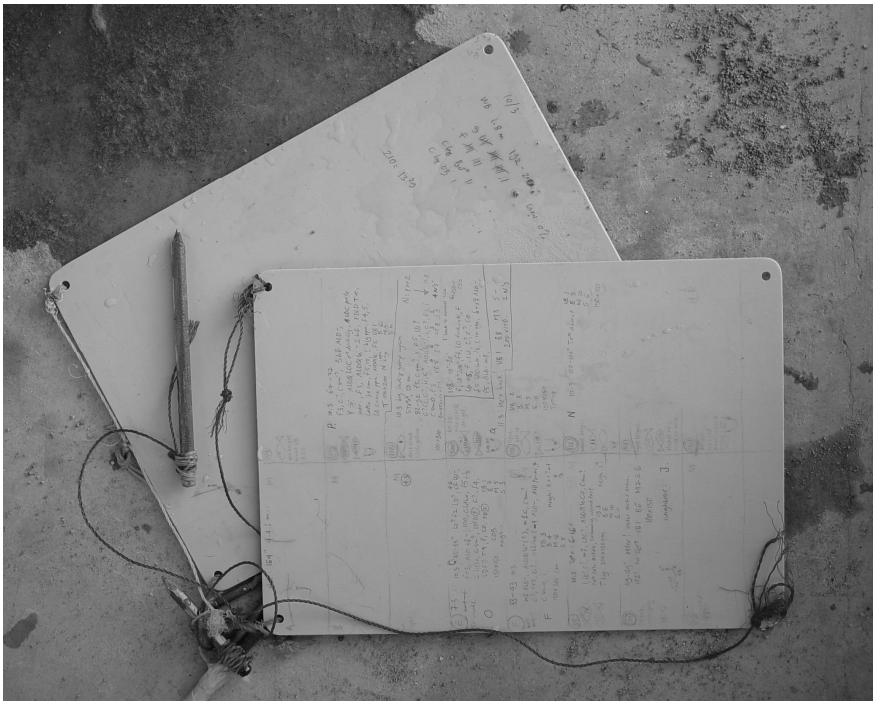
Chapter 5

'Not only are the laws of inheritance extremely complex, but so are the causes which induce and govern variability. The variations thus induced are preserved and accumulated by sexual selection, which is in itself an extremely complex affair, depending, as it does, on the ardor in love, the courage, and the rivalry of the males, as well as on the powers of perception, the taste, and will of the female.'

Darwin, 1871



Sensory drive in cichlid speciation

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The role of selection in speciation is a central yet poorly understood problem in evolutionary biology. The rapid radiations of extremely colourful cichlid fish in African lakes have fuelled the hypothesis that sexual selection can drive species divergence without geographical isolation. Here we present experimental evidence for a mechanism by which sexual selection becomes divergent: in two sibling species from Lake Victoria, female mating preferences for red and blue male nuptial coloration coincide with their context-independent sensitivities to red and blue light, which in turn correspond to a difference in ambient light in the natural habitat of the species. These results suggest that natural selection on visual performance, favouring different visual properties in different spectral environments, may lead to divergent sexual selection on male nuptial coloration. This interplay of ecological and sexual selection along a light gradient may provide a mechanism of rapid speciation through divergent sensory drive.

Introduction

It is widely acknowledged that female mate choice for male ornamental traits can maintain and strengthen reproductive isolation between recently diverged species, even without postzygotic barriers to gene flow (Andersson 1994). The role of sexual selection in the emergence of reproductively isolated populations, hence in the speciation process itself, is less well understood and much more controversial (Arnegard & Kondrashov 2004). Especially when female choice selects for 'good genes', it is hard to see how variation in mating preferences can evolve, let alone drive species divergence (Kirkpatrick & Nuismer 2004, but see Edelaar et al. 2004; Reinhold 2004). However, intraspecific variation in female preferences has been demonstrated in several taxa (e.g. Godin & Dugatkin 1995; Coleman et al. 2004), and more examples may emerge as research effort into the evolution of signal perception is increasing (Jennions & Petrie 1997).

The extremely species rich cichlid communities of the East-African Lakes Malawi and Victoria have been presented as possible examples of speciation driven by sexual selection (Dominey 1984; McKaye 1991; Seehausen 2000; Danley & Kocher 2001). This hypothesis was inspired by the widespread occurrence of intra- and interspecific variation in male nuptial coloration and corresponding female mating preferences, often associated with only slight ecological differentiation and no intrinsic postzygotic isolation between species (Albertson et al. 1999; Seehausen 2000).

Haplochromine cichlids express considerable interspecific variation in colour vision (Van der Meer & Bowmaker 1995; Smit & Anker 1997). Seehausen et al. (1997a) proposed that if such variation occurred within populations, together with directional sexual selection by female mate choice for brightly coloured, conspicuous males, it might constitute disruptive sexual selection on male coloration and possibly cause speciation. Several recent studies have presented evidence for divergent sensory drive in species pairs that use visual signals in sexual communication (Boughman 2001; Fuller 2002; Leal & Fleishman 2004), indicating that it may be a potent driving force in speciation (Endler & McLellan 1988; Endler et al. 2001; Boughman 2002).

We investigate the divergence of a species pair that represents the dominant pattern of colour variation among sympatric closely related Lake Victoria cichlids (Seehausen et al. 1999c). *Pundamilia pundamilia* and *Pundamilia nyererei* are anatomically similar, but *P. pundamilia* males are metallic blue and *P. nyererei* males are bright red (Figure 5.1a). They behave as reproductively isolated species in some populations, but interbreed in locations where the water is very turbid (Seehausen et al. 1997a). Females of both species are cryptically coloured and can be distinguished only with difficulty. They have assortative mating preferences and use male coloration as a choice criterion (Seehausen & Van Alphen 1998). The hypothesis that sensory drive has been involved in the divergence of this species pair (Seehausen et al. 1997a) predicts that the photic environments in the microhabitats of the two species differ, that the visual systems of the two species have

diverged in adaptation to these different photic environments, and that, within a species, females prefer more conspicuous over less conspicuous males. The latter has been demonstrated in one of the two species: the red coloration of *P. nyererei* males is subject to directional sexual selection by female choice (Chapter 2).

Here we report data consistent with divergent adaptation of the visual systems of the two species. First, we quantify the photic habitats of both species in nature. Although geographically, *P. nyererei* is entirely sympatric with *P. pundamilia*, it breeds in deeper water (Seehausen & Bouton 1997). Because the turbid Lake Victoria waters selectively absorb and scatter light of short wavelengths, the intensity and spectrum of ambient light rapidly change with depth (De Beer 1989). Second, we test whether the two species differ in visual properties, in the direction predicted by the difference in spectral environment. We use the optomotor response test to measure the context-independent behavioural responses to coloured light of both species.

Methods

Fish

Fish were collected in February 2003 at Makobe Island (Seehausen & Bouton 1997) in the western Speke Gulf of Lake Victoria (Tanzania; water transparency: Secchi reading mean±se=221±7 cm; 84 measurements in 2000-2003). At this island, *P. pundamilia* and *P. nyererei* inhabit a continuous rocky slope from 0.5 to 7 meters water depth. *P. pundamilia* is most abundant at 0.5-2 meters depth; *P. nyererei* is most abundant at 4-7 meters depth. Both species occur at 2-4 meters depth (Seehausen & Bouton 1997; pers. obs.). Their main habitats are not separated by any barrier (such as stretches of sand or mud), nor are they isolated by distance (distances between locations within each habitat are not shorter than those between the habitats). Individuals of both species are regularly observed and caught in the entire 0.5-7 m depth range. Sensitivity thresholds were determined in October-December 2003. As we were interested in correspondence between visual sensitivity and female preference, we used females only. *P. nyererei* females (n=11) were caught in gillnets at 4-7 m depth; *P. pundamilia* females (n=14) were collected using hook and line at ~1 m depth. Fish were shipped to the Netherlands and kept at 25±2°C and a 12:12 photoperiod. Tanks were illuminated with high frequency fluorescent light tubes (Philips TLD 58 W, light colour 830, 1100 lux). Shortly before an experiment, we measured fish standard length and inserted a microchip into the abdominal cavity for individual identification (12 mm glass tags, UKID122GL, Biomark Inc.); species identity of the subjects was not known to the observer during the experiments.

Spectrophotometry

Reflection spectra of male nuptial coloration were measured using an Avantes PS 2048 spectrophotometer with SpectraWin 6.1 software (Avantes). We used one sexually active male of each species and measured reflectance in daylight in clear

water, holding the probe of the spectrophotometer at a distance of 5 mm perpendicular to the fish skin and holding the fish under water. Light reflectance was calibrated using a dark reference (covering the probe completely) and a light reference (reflectance off a white piece of plastic, also under water); no additional illumination was used. For *P. pundamilia*, we measured reflectance off the blue sides of the fish; for *P. nyererei*, we measured the red dorsum (which is more important in female choice than the yellow coloration on the sides of the males; Chapter 2). We normalised reflectance spectra by the peak reflectance to facilitate species comparison.

Treatment light spectra were measured using the same equipment and holding the probe in the middle of the experimental tank, illuminated by the treatment light and filled with water to 15 cm. With decreasing light intensity, the spectral curves became less steep but the wavelength of maximum intensity did not change (wavelength of top 5% intensity [mean±se] blue: 447.9±1.3 nm; red: 670.2±0.7 nm).

We measured light transmission at Makobe Island with an Ocean Optics PS 1000 spectrophotometer with a 10-meter optical fibre cable (100 micron) and using SpectraWin 4.16 software (Avantes). We quantified the spectral composition at different depths by calculating the 'orange ratio': the light intensity in the 550-700 nm range (yellow, orange, red) divided by the intensity in the 400-550 nm range (blue, green)(Endler & Houde 1995).

Optomotor experiment

The optomotor response test exploits the optokinetic response: the tendency of fish to follow a moving light stimulus. The method has been successfully employed in a variety of fish species (e.g. guppies: Anstis et al. 1998, zebrafish: Krauss & Neumeyer 2003, goldfish: Schaerer & Neumeyer 1996, sticklebacks: Boughman 2001, and cichlids: Van der Meer 1994; Kröger et al. 2003. Fish were placed individually in a cylindrical tank (diameter 20 cm, water depth 15 cm; Figure 5.1d). A screen with vertical black and white bars (35 mm width) rotated around the tank at 8.33 rpm. Trials started with an acclimatisation period of 10 minutes during which the bar pattern rotated and the tank containing the test fish was illuminated with a small adapting light (230 lux), an infrared light source (875 nm, intensity 17 mW/sr, Agilent Technologies) above the tank, and the maximum intensity of treatment light. Treatment light (Figure 5.1b) was provided by a slide projector (GAF 131) with either a blue (436 nm) or a red (656 nm) narrow band filter (Spindler & Hoyer). To narrow wavelength ranges, we added a coloured Lee filter (113, magenta) and density filters (Lee 209; one density filter for the blue treatment and two density filters for the red treatment). Light intensity was adjusted in 18 discrete steps by lowering the voltage feeding of the slide projector (Figure 5.1c and e). The response of the fish was observed using an infrared camera with monitor. After the habituation period, the adapting light was switched off. If the fish continued following the rotating bar pattern within 15 seconds for at least 10 seconds, the treatment light intensity was lowered by one step. This was repeated

until the swimming response had disappeared. To confirm this threshold, the light intensity was increased by two steps, and again lowered stepwise, this time reversing the rotating direction once at each light intensity level. Sensitivity threshold was defined as the lowest light intensity at which the fish responded to reversal of the rotation direction by reversing the swimming direction. The sensitivities that could be measured therefore ranged from zero (maximum light intensity) to 18 (minimum light intensity). During the test procedure, the adapting light source was switched on for one minute every 90 seconds, to ensure the fish were light-adapted (Smit & Anker 1997). Each individual was tested five times in each light treatment and the median sensitivity threshold of the test series was used for statistical analysis.

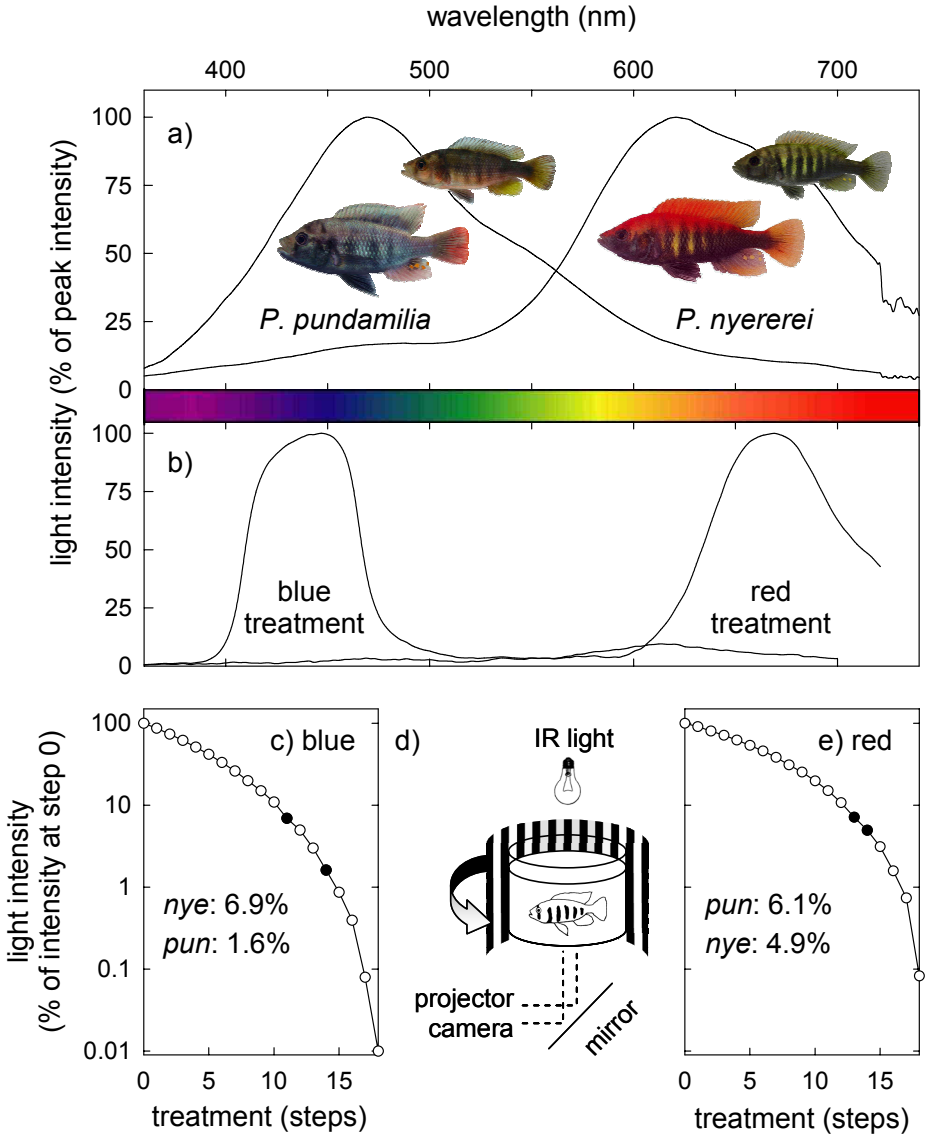

Figure 5.1 Male nuptial coloration in *Pundamilia* and the light treatments in the optomotor response test. a) *P. pundamila* and *P. nyererei* males and females with the reflection spectra of male nuptial coloration. b) Transmission spectra of the blue and red light treatments used in the optomotor experiment. c) and e) Treatment light intensities, from step 0 to 18, as a percentage of the treatment light intensity in step 0. Group medians for both species (black symbols) are given in each panel. d) The experimental setup.

Data analysis

Species differences were analysed using independent samples t-tests (normal data) and Mann-Whitney-U tests (non-normal data) in SPSS 10.0 (SPSS Inc.). To disentangle the effects of body size and species identity we analysed generalised linear models (GLM) in R (http://www.r-project.org; Ihaka & Gentleman 1996) with a Poisson distribution. Also in R, the orange ratio at Makobe Island was analysed in GLMs with binomial distribution. Significance was determined by F-tests examining the change in deviance following removal of variables. Test statistics were adjusted for over- and underdispersion (Venables & Ripley 2002).

Results

The photic environment

At Makobe Island, the proportion of long wavelengths (i.e., >550 nm) in the light spectrum increased rapidly with depth ($F_{1,9}$=24.9, p=0.0008; Figure 5.2). As a result, the light spectrum in the habitat where *P. nyererei* dominates (4-7 m depth) was significantly more red-shifted (orange ratio: 0.75) than that in the habitat where *P. pundamilia* is most abundant (0.5-2 m depth; orange ratio: 0.57): $F_{1,4}$=8.79, p=0.04.

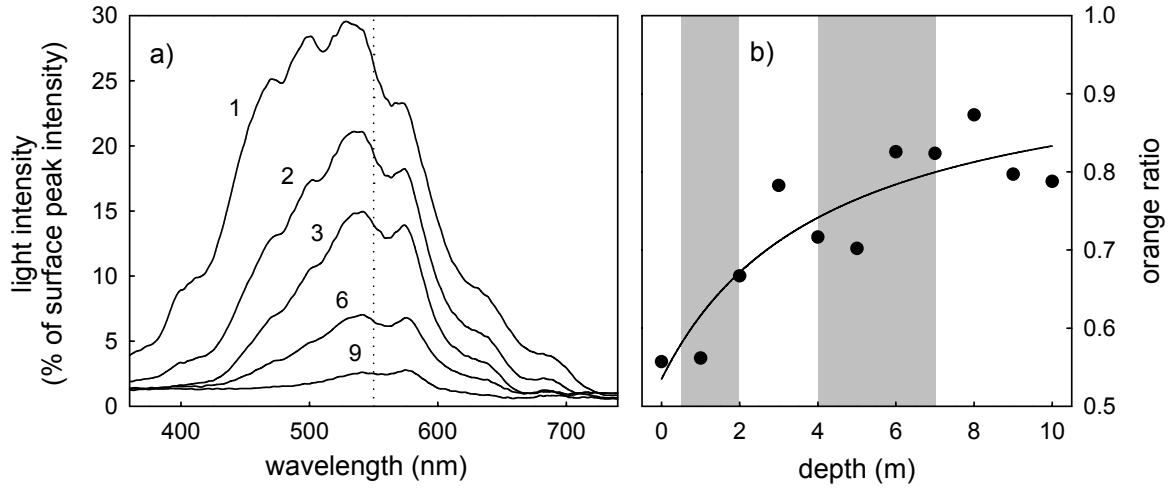

Figure 5.2 Photic habitats in the study population at Makobe Island. a) Transmission light spectra at different depths. Numbers indicate depth in meters. The dotted line indicates the 550 nm threshold which is used to calculate the orange ratio. b) The increase in orange ratio with water depth. Shaded areas indicate *P. pundamilia* (0.5-2 m) and *P. nyererei* (4-7 m) breeding habitats.

The sensitivities to coloured light

In both blue and red light, there were significant differences in optomotor response between the two species (Figure 5.3). In blue light, *P. pundamilia* females performed significantly better than *P. nyererei* females (n_1=14 n_2=11, *MWU*

Z=2.86, p=0.004). In red light, the pattern was reversed (Z=2.53, p=0.011). The minimum intensity of blue light that *P. pundamilia* responded to was 2.7 % of (or 37.7 times lower than) the minimum intensity of blue light that *P. nyererei* responded to. The minimum intensity of red light that *P. nyererei* responded to was 23.7 % of (or 4.2 times lower than) the minimum intensity of red light that *P. pundamilia* responded to. Together, the blue and red optomotor response thresholds categorized 23 out of the 25 individuals tested into two non-overlapping groups, one with individuals having high sensitivity to red light and low sensitivity to blue light (all *P. nyererei*) and the other with individuals having high sensitivity to blue light an low sensitivity to red light (all *P. pundamilia*). Thus, if mate choice in nature corresponds to visual sensitivities as measured by optomotor response thresholds, these results are consistent with assortative mating for 23/25 individuals (92%).

On average, *P. pundamilia* females were larger than *P. nyererei* females (mean±se, standard length [mm]: *P. pundamilia*: 84.7±1.1 and *P. nyererei*: 79.7±1.1, independent samples t-test: t_{23}=3.11, p=0.005; weight [g]: *P. pundamilia*: 18.9±0.7 and *P. nyererei*: 13.8±0.5, t_{23}=5.48, p<0.001). The performance in the experiment was not correlated with body size in either species (Spearman rank correlations between weight and standard length and red and blue sensitivity: all r_s<0.41, p>0.21). GLMs including species, standard length and weight showed that sensitivity threshold was best explained by species identity (red: estimate [species]= 0.11±0.036, $F_{1,23}$=9.47, p=0.0053; blue: estimate [species]= -0.23±0.061, $F_{1,23}$=14.11, p=0.001). Models did not improve by including body size information (all $F_{1,22}$<0.062, p>0.81 for the difference between models).

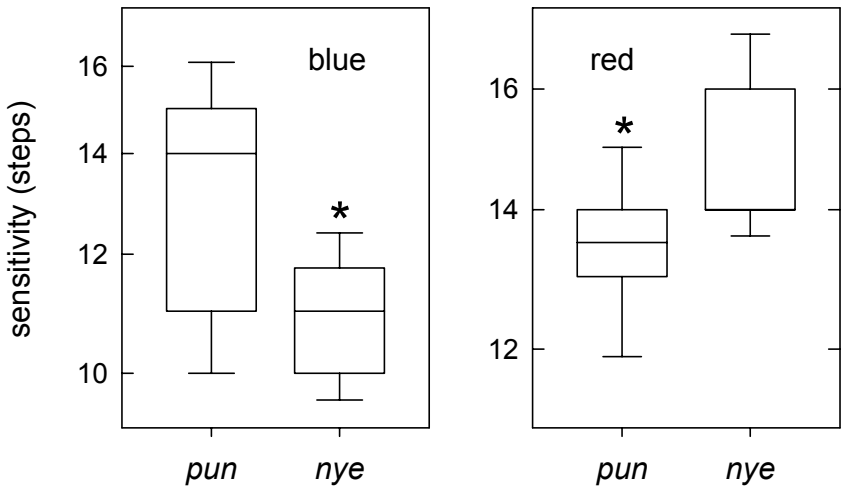

Figure 5.3 The performance of *P. pundamilia* (*pun*, n=14) and *P. nyererei* (*nye*, n=11) females in the optomotor response test. Left panel: blue light; right panel: red light. Boxes are medians with first and third quartiles, error bars indicate 10th and 90th percentiles. Asterisks: p<0.02.

Discussion

We investigated the hypothesis that divergent natural selection on visual properties has contributed to the divergence of female mating preferences between sibling species of Lake Victoria cichlid fish, *P. pundamilia* and *P. nyererei* (Seehausen et al. 1997a). This hypothesis was inspired by the observations that the species differ strikingly in male nuptial coloration; that the cryptically coloured females use this difference as a basis for species-assortative mate choice (Seehausen & Van Alphen 1998), and that female mate choice exerts directional sexual selection on male coloration in at least one of the two species (*P. nyererei*, Chapter 2). Moreover, the spectral characteristics of the Lake Victoria waters create a heterogeneous light environment (De Beer 1989) with potentially steep selection gradients.

We quantified the spectral habitats of *Pundamilia pundamilia* and *Pundamilia nyererei* at Makobe Island, and found that the two species indeed inhabit significantly different light environments. Compared to the shallow-breeding *P. pundamilia*, *P. nyererei* experiences a narrower and red-shifted ambient spectrum. In optomotor experiments, we tested whether this difference in light environment coincides with a difference in sensitivity to coloured light. Our data demonstrate that the species indeed differ in behavioural responses to colour stimuli outside the mate choice context: *P. pundamilia* females responded to lower intensities of blue light than did *P. nyererei* females. Conversely, *P. nyererei* females responded to lower intensities of red light than did *P. pundamilia* females.

Since we used wildcaught fish, collected from different spectral environments, the differences we found could be due to phenotypic plasticity (Kröger et al. 2003). There are however strong indications for heritable differences in the visual systems of the two species. First, *P. nyererei* has a larger number of long-wavelength sensitive (LWS) cones than *P. pundamilia* and this difference is maintained in fish that are bred in identical laboratory conditions (Carleton et al. 2005). Second, the gene sequence of the LWS opsin gene differs between the species, corresponding to a 3-4nm shift of maximum absorbance towards longer wavelengths in *P. nyererei* (Carleton et al. 2005).

Several earlier studies have demonstrated differences in absorbance spectra between cone pigments extracted from retinas of different haplochromine species (Van der Meer & Bowmaker 1995), in cone opsin gene and protein sequences (Carleton & Kocher 2001; Terai et al. 2002), and in opsin gene expression (Carleton & Kocher 2001). Our study provides the first evidence that differences between sibling species in opsin protein sequences, opsin expression patterns (Carleton et al. 2005) and female mating preferences for different male colours (Seehausen & Van Alphen 1998), are accompanied by differences in context-independent behavioural responses to light of different wavelengths. This is an important but previously untested prediction of the sensory drive speciation hypothesis.

Many animals use only long-wavelength sensitive (LWS) cones for the perception of movement (e.g. goldfish: Schaerer & Neumeyer 1996) and this may

hold for cichlid fish as well (but see Kröger et al. 2003). In the case of *Pundamilia*, the difference in optomotor response between *P. pundamilia* and *P. nyererei* could be due to differences in expression level or in the peak sensitivity of a single cone pigment, most likely LWS (Carleton et al. 2005). By contrast, colour assessment in the mate choice context may involve the joint action of all cone types (three different cone pigments). Further, behavioural responses to visual stimuli are mediated by information processing in the brain. For these reasons, further work is required to investigate how variation in *Pundamilia* optomotor responses relates to variation in female preferences for male nuptial coloration.

Either way, our data are consistent with the hypothesis that the visual systems of *P. pundamilia* and *P. nyererei* have evolved under divergent natural selection exerted by the spectral depth gradient in the natural habitat of the fish, and that this in turn mediates divergent or disruptive sexual selection on male nuptial coloration. Increased sensitivity to light of longer wavelengths (longer wavelength of maximum sensitivity in the LWS cones, i.e. longer LWS λ_{max}, and/or a larger proportion of LWS-expressing cones) is advantageous in deeper water, while shorter LWS λ_{max} and a larger proportion of short-wavelength sensitive (SWS) expressing cones are favoured in shallow water. Consequently, if females prefer conspicuous males (Chapter 2; Pauers et al. 2004), males that reflect mainly on the blue end of the spectrum have an advantage in shallow water where selection favours shorter λ_{max} and a larger proportion of SWS expressing cones. In deeper water, where selection favours longer λ_{max} and a larger proportion of LWS expressing cones, red males are likely to have higher mating success.

Fish visual systems tend to be adapted to the spectral environment (Ali 1975; Loew & Lythgoe 1978; Levine & MacNichol Jr. 1982; Van der Meer & Bowmaker 1995; Cummings & Partridge 2001; Jokela et al. 2003). The relationship between spectral environment and sexual colour signals however is often negative (Marchetti 1993; Boughman 2001; Fuller 2002; Leal & Fleishman 2004): wavelengths that dominate in the ambient light spectrum are not used in visual signals. This maximises colour signal conspicuousness through background contrasting. In *Pundamilia* however, male nuptial coloration exploits the available extremes in the ambient spectrum to maximise contrast against the green-yellow background light (Figure 5.2). Thus, the blue *P. pundamilia* inhabits a broad-spectrum environment where blue wavelengths are available to generate contrast. The red *P. nyererei* inhabits a red-shifted photic environment and exploits the long-wavelength extreme of the ambient spectrum to maximise contrast against the background light. This difference with other taxa may be due to the steepness of the spectral gradient in Lake Victoria. In the *P. nyererei* habitat at Makobe Island, light intensity in the short wavelength range is so low that blue signals are not conspicuous or even invisible there.

We conclude that the interaction of divergent ecological selection on the visual system along a spectral light gradient and directional sexual selection for bright male colour may result in divergent or disruptive sexual selection on male coloration through female mate choice. Consistent with sensory drive speciation

models (Endler 1992; Boughman 2002), this mechanism may have driven the divergence of *P. pundamilia* and *P. nyererei*. The geographically sympatric and ecologically parapatric distribution of the two species suggests that this process may have occurred without geographical isolation. The same mechanism is also likely to contribute to the maintenance of reproductive isolation after speciation. Given the abundance of blue-red sister species and polymorphisms among Lake Victoria cichlid fish (Seehausen et al. 1999c), we postulate that similar mechanisms may have been involved also in other cases of rapid speciation that resulted in sympatric sister species which differ in male nuptial coloration and depth distribution. Likewise, divergent selection on visual properties may have contributed to the cichlid radiations in other African lakes. In Lake Malawi for example, which has a higher water transparency and shorter wavelength of maximum transmission than Lake Victoria, blue-yellow rather than blue-red species pairs or morphs predominate (McElroy et al. 1991; Deutsch 1997; Seehausen et al. 1999c). This corresponds to patterns of opsin gene expression in these fish (Carleton & Kocher 2001).

Acknowledgements

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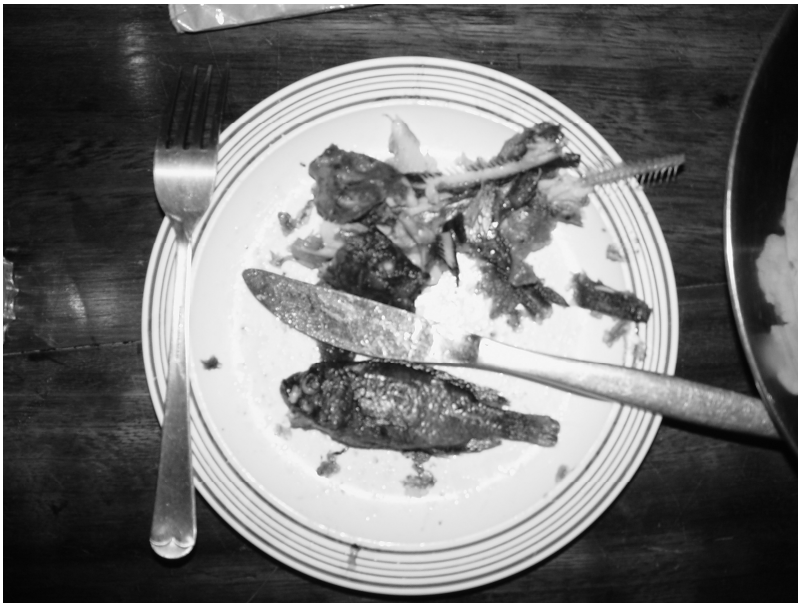
Chapter 6

'The more diversified the descendants from any one species become in structure, constitution, and habits, by so much will they be better enabled to seize on many and widely diversified places in the polity of nature, and so be able to increase in numbers.'

Darwin, 1859



Female mating preferences and male coloration co-evolve in response to water transparency in a Lake Victoria cichlid fish

Martine E. Maan, Ole Seehausen and Jacques J.M. van Alphen

(submitted)

The Lake Victoria cichlid species flock is a classic example of rapid speciation that may be driven by sexual selection: female choice for male nuptial coloration exerts sexual selection within species and maintains reproductive isolation between species. Recently however, water transparency in Lake Victoria has been declining, with critical consequences for cichlid diversity: low water transparencies coincide with increasing rates of hybridisation between closely related species, decreasing species numbers and less colourful fish. Here, we investigate a possible mechanism underlying this pattern: increased turbidity may lead to relaxation of sexual selection on male nuptial coloration within species, affecting both male nuptial coloration and the rate of interspecific hybridisation. We study *Pundamilia nyererei*, a species known to interbreed with a sister species in turbid water, but not in clear water. We compare measures of intraspecific sexual selection between two populations from locations that differ in water transparency. In clear water, female *P. nyererei* exert directional sexual selection on male red coloration. Here we find that in the population from turbid water, female preference for male red coloration is significantly weaker. Further, we find that both the amount and the hue of male red coloration differ significantly between populations, consistent with adaptation to the different photic habitats of the two populations. These findings suggest that the observed correlation between male coloration and water transparency is not mediated by environmental variation alone. Rather, they indicate that female mating preferences have evolved in response to this variation. This is the first evidence for intraspecific preference-trait co-evolution in cichlid fish. Our findings underline the importance of measures to counteract the ongoing eutrophication of Lake Victoria.

Introduction

Animal communication strongly depends on the signal propagation properties of the environment. Animals have been shown to adjust their sexual signals accordingly, trading off different components of sexual display in response to environmental variation. Differential employment or elaboration of visual, acoustic, seismic or behavioural displays has been demonstrated both at the intraspecific (e.g. spiders: Taylor & Jackson 1999) and interspecific level (e.g. birds: Badyaev et al. 2002). Also human-induced changes in the environment influence the nature and effectiveness of animal signals (Seehausen et al. 1997a; Slabbekoorn & Peet 2003).

In water, visual communication is constrained by the light transmission properties of the water and its organic and inorganic content: water turbidity can severely hamper sexual selection on visual cues (Järvenpää & Lindström 2004). Besides overall turbidity, the wavelength distribution of the transmitted light affects the visibility of colour signals. First, the ambient light spectrum determines the colour of the background against which a signal is observed, influencing its conspicuousness (e.g. Marshall 2000; Fuller 2002). Second, the spectrum determines the 'colour space' available for reflection and perception (Seehausen et al. 1997a). In guppies and sticklebacks, male colour traits and female preferences for these traits co-vary across populations that differ in water colour (Endler & Houde 1995; Boughman 2001).

In Lake Victoria cichlid fish, female mate choice for male nuptial coloration plays an important role in the evolution and maintenance of an exceptional species diversity (Dominey 1984; Seehausen 2000). Due to human population growth that entails increased deforestation and nutrient runoff, water transparency in the lake has been declining for several decades (Verschuren et al. 2002). Because many haplochromine cichlids rely on visual cues for mate recognition (Seehausen & Van Alphen 1998; Knight & Turner 2004), increased turbidity poses a threat to species diversity (Seehausen et al. 1997a).

Pundamilia pundamilia and *Pundamilia nyererei* are two closely related species that occur sympatrically at several islands in south-eastern Lake Victoria. They are morphologically similar, but differ in male nuptial coloration: male *P. pundamilia* are metallic blue and male *P. nyererei* are bright red and yellow. Females of both species are cryptically coloured. Reproductive isolation is maintained by female choice for different male nuptial coloration (Seehausen & Van Alphen 1998), but the two phenotypes hybridise in locations with low water transparency. In extremely turbid water (Secchi disk reading below 50 cm), populations consist of hybrids only (Seehausen et al. 1997a). Further, the brightness of *P. nyererei* male nuptial coloration decreases as the water becomes more turbid (Seehausen et al. 1997a).

The mechanism by which transparency affects male coloration is unknown: i) female mating preferences may evolve in response to variation in water transparency, or ii) the strength of selection on male coloration by female mating preferences may be a direct function of signal transduction through the water, without

mating preferences evolving themselves. Here, we investigate these alternatives. Previous work has shown that in a clear water population of *P. nyererei*, female choice exerts directional sexual selection on male red coloration (Chapter 2). In the present study, we investigate the mate choice behaviour of a *P. nyererei* population from turbid water, using the same laboratory set-up as before to allow for comparison of the two populations. To test whether female choice, male coloration and ambient light spectrum covary, we measure the light spectrum in both habitats and we quantify the variation in male coloration in both populations.

Methods

Fish

Male *Pundamilia nyererei* are bright red dorsally and have yellow sides intercepted by black vertical bars. Females are cryptically brown with dark vertical bars. We compare *P. nyererei* populations from two islands in south-eastern Lake Victoria that differ in water transparency: Kissenda (Secchi disk reading mean±se =67.5±1.4 cm, n=4, in the study period) and Makobe (221±15 cm; n=29, Figure 6.1a). Hybrids between *P. nyererei* and *P. pundamilia* have never been observed at Makobe Island, but are regularly encountered at Kissenda (in 2001-2003, we caught 1 hybrid male with every 15 *P. nyererei* males, n=79 fish, unpublished data; see also Taylor et al. subm.). At Makobe, *P. nyererei* territories are most abundant between four and seven meters depth and fish were collected by gillnetting. At Kissenda, *P. nyererei* predominantly occurs between two and five meters depth and fish were collected by gillnetting and hook and line. In both populations, fish were collected between December 2000 and April 2001 and mate-choice experiments were carried out between November 2001 and August 2002. In the lake, *P. nyererei* males defend territories on the rocky bottom and attract females by vigorous courtship displays that resemble those of other haplochromine species (See-

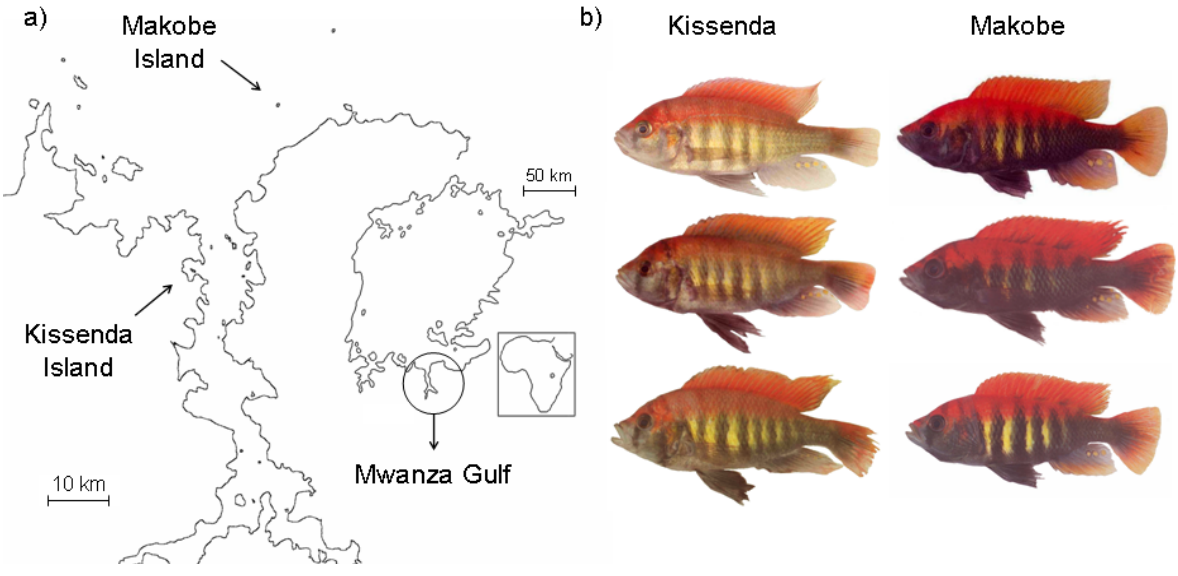


Figure 6.1 a) Lake Victoria and the locations of Kissenda and Makobe Islands.
b) *Pundamilia nyererei* males from both islands.

hausen & Van Alphen 1998). Courtship typically starts with Lateral Display, in which the male positions itself perpendicular to the female and spreads all fins. This is followed by Quiver, a high-frequency shaking movement of the body. Finally, the male leads the female to the centre of the territory in a quick swimming bout, often with exaggerated tail beats (Lead Swim). Mating occurs in rocky crevices. After spawning, females mouthbrood eggs and larvae for about three weeks and guard the fry for an additional week after releasing them (Seehausen 1996).

Spectrophotometry

Light transmission spectra were determined in the 350-750 nm range, using an AvaSpec 2048 spectrophotometer with a 10-meter fibre cable (100 micron) and SpectraWin 4.16 software (Avantes). Measurements were taken in the shadow, between 8h50 and 9h00 in the morning, on November 26th 2002 at Kissenda Island and on December 1st 2002 at Makobe.

Mate choice experiment in the laboratory

Two-way mate choice experiments were carried out following the methods of Chapter 2: each male was kept in one of two six-sided perspex enclosures (140 litres) on either side of an 1800-litres aquarium. In each enclosure, shelter was provided by a brick on top of a PVC tube. These shelters were readily accepted by the males as the centres of their territories. In the middle of the main tank two large stones provided shelter for the test female and ensured that the males could not see each other. The water in the male enclosures was filtered internally, making chemical communication impossible. Air stones were present in both enclosures and in the main tank; water temperature was maintained at 24-26 °C.

We used 10 wildcaught *P. nyererei* males from Kissenda Island, assembled into 5 male pairs of fixed composition, and 9 Kissenda females. All males were photographed, measured and weighed. Redscore differences (see below) between Kissenda males in a pair ranged from 3 to 12% body coverage. Four Makobe males were used to assemble two additional pairs with large redscore difference: 9% and 24.8% body coverage. Size differences between paired males were below 7% of the average size of the two males in a pair.

Males were released into their compartments the evening before trials. A maximum of three females was tested with one male pair in a day and at least two hours separated consecutive trials. Male pairs were exchanged after three successful trials or after two days. The position of the males (left or right side of the aquarium) was reversed each time the same pair was introduced. To start a trial, the test female was released in the middle of the tank. Behaviour was recorded with Observer 3.0 software (Noldus). Observation time started when the test female was within 30 cm of either one of the male compartments and stopped when she left this area. This distance was chosen because, in field observations, any fish within 30 cm of a *P. nyererei* territory elicits a behavioural response of the territory owner, indicating 30 cm as an interaction threshold distance. Trials were completed once 15 minutes of observation time had been collected.

Each female was tested once with every male pair, but in the analysis we included only those trials in which both males courted (performing Quiver or Lead Swim at least once) and the test female responded positively (approaching a male in response to his Lateral Display, Quiver or Lead Swim) to at least one of them (32 trials with Kissenda males; 11 trails with Makobe males). For every male courtship display event, we recorded whether the female responded by approaching the male. The resulting proportion is our measure of female response to each male. The difference in female response to two males in a trial is our measure of female preference. We also recorded aggressive behaviour of the males (butting and biting attempts) and counted the total number of encounters of each male with the test female. The results of this experiment are compared to a previous mate-choice experiment carried out with Makobe males and females (Chapter 2).

Male coloration at Makobe and Kissenda Islands

To quantify male colour variation in the field, we collected mature males from both islands. At Makobe Island, we collected 28 males that were territorial and sexually active, as established during observations using SCUBA. Water transparency at Kissenda Island does not allow for underwater observations. Therefore, we collected 17 males that expressed red, yellow and black nuptial coloration and that belonged to the largest 10% of the population. Immediately after capture, males were photographed for colour analysis and sacrificed on melting ice. They were subsequently measured (standard length, SL, to the nearest 0.1 mm) and weighed (W, to the nearest 0.1 g). We calculated condition factor (CF) as $CF=100\cdot(W/SL^3)$ (Sutton et al. 2000).

Colour analysis

For photography, males were placed in a perspex cuvette with water and gently squeezed against the front window of the cuvette with a grey PVC sheet. We used an SLR camera and two flashes on either side and subsequently digitised pictures. We adjusted white balance in PhotoShop 6.0 (Adobe Systems Inc.) using a white patch (Kodak colour card) attached to the front of the cuvette. We calculated colour scores in SigmaScan Pro 4.0 (SPSS Inc.). We defined criteria delimiting the body area (excluding fins and eyes) covered by red and yellow by a combination of hue (RGB) and saturation (red: hue = 0-26 plus 232-255, saturation 40-97%; yellow: hue = 27-45, saturation 40-97%) and subsequently calculated the area of the fish body that matched these criteria, yielding a percentage of body coverage, subsequently referred to as redscore and yellowscore. We similarly defined criteria for blackness to quantify the body coverage of the black vertical bars and ventral aspects of the body (intensity=0-75; blackscore). To compare male coloration between populations, we calculated an additional variable: dorsal hue. This is the average hue of the male dorsum, measured in five standardised positions, using the colour sampler tool in Adobe PhotoShop 6.0. Hue was defined as a location on the standard colour wheel, expressed as a degree between 0 and 360, where 0° corresponds to red and 60° corresponds to yellow.

Data analysis

Male and female behaviour in the mate choice experiments was analysed in generalised linear models (GLM) using R software (Ihaka & Gentleman 1996; http://www.r-project.org). We separately report the results for the three most important male courtship displays: Lateral Display, Quiver and Lead Swim. Female response to male courtship was analysed in Poisson models with log link functions. To compare female response between the two males in a pair, we used binomial models with logit link functions. We tested whether females had significant preferences for those males in the male pairs that were largest (standard length) or that had the highest colour scores (red, yellow, black). For each of these male characters, we also tested whether female preference increased with increasing size or colour difference between the males in a pair. To control for random effects of individual females and male pairs, we included 'trial number' as a factor in all models. To compare the two populations, we added 'population' as a factor in the models. Significance levels were determined by F-tests examining the change in deviance following removal of a variable; test statistics were adjusted for over- and underdispersion (Venables & Ripley 2002).

Other statistical analyses were carried out in SPSS 10.0 (SPSS Inc.), using t-tests and Pearson correlations for normally distributed data, and Mann-Whitney-U tests and Spearman correlations for non-normally distributed data.

Results

Behaviour of Kissenda females

In the trials with Kissenda males and females, females showed a significant preference for the male in a pair with the highest redscore (Figure 6.2; Lateral Display estimate=0.819±0.149, $F_{1,31}$=30.93, p<0.0001, Quiver estimate=0.831±0.186, $F_{1,31}$=20.41, p<0.0001, Lead Swim estimate=1.057±0.311, $F_{1,26}$=12.23, p= 0.0017). Female preference did not increase with increasing redscore difference between the males (Lateral Display and Lead Swim: F<2.31, p> 0.14); differential

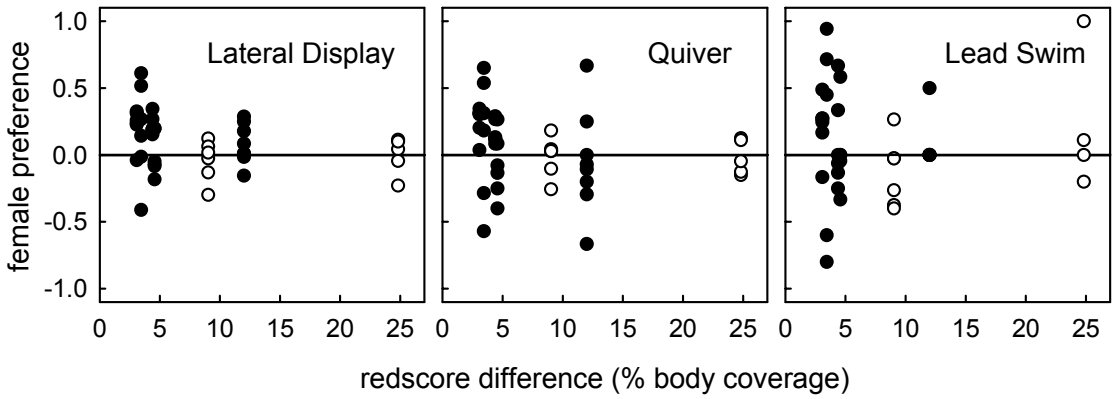


Figure 6.2 Female preference relative to the difference in redscore between the males in a pair, for each of the three courtship displays. Each symbol represents one trial; filled circles are trials with Kissenda males; open circles are trials with Makobe males.

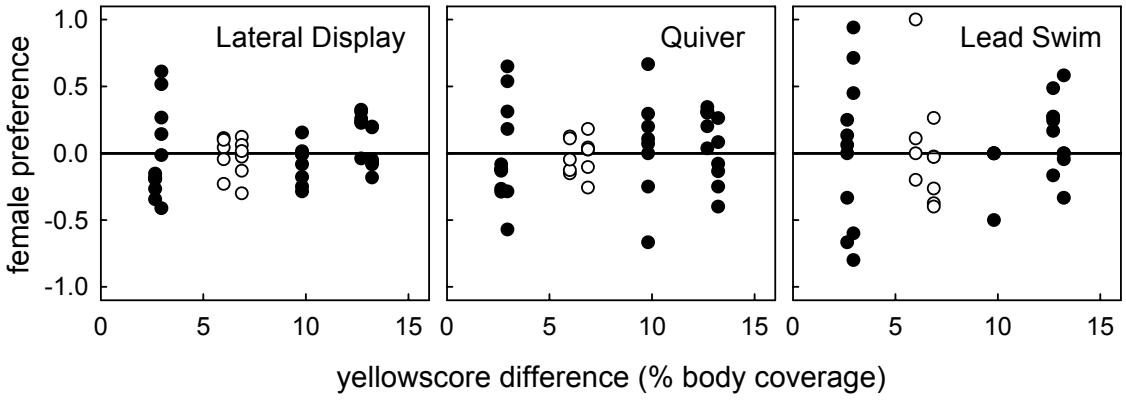


Figure 6.3 Female preference relative to the difference in yellowscore between the males in a pair, for each of the three courtship displays. Each symbol represents one trial; filled circles are trials with Kissenda males; open circles are trials with Makobe males.

response to male Quiver decreased with increasing redscore difference (estimate=-21.36±7.82, $F_{1,30}$=7.88, p=0.009).

The differences in colourscores and standard length between males in a pair were not correlated (Spearman correlations, r_s<0.6, p>0.10). Yet, females also significantly preferred the males with the highest yellowscores (Figure 6.3; Lateral Display estimate= 0.400±0.195, $F_{1,31}$=4.24, p=0.048, Quiver estimate= 0.736±0.200, $F_{1,31}$=13.92, p<0.001, Lead Swim estimate= 1.034±0.298, $F_{1,26}$=12.05, p=0.0018). For Lateral Display, there was a non-significant tendency for female preference to increase with increasing yellowscore difference between the males (estimate=7.500±4.039, $F_{1,30}$=3.44, p=0.073). For Quiver and Lead Swim, there were no such trends (F<1.38, p>0.25).

There were no significant preferences for male blackscore (F<1.42, p>0.24 for all displays). Finally, females responded more strongly to the Lateral Display of the larger male in a pair (estimate=0.470±0.190, $F_{1,31}$=6.15, p=0.019) but this was not significant for Quiver (estimate=0.442±0.222, $F_{1,31}$=3.99, p=0.055) and Lead Swim (estimate=0.185±0.349, $F_{1,26}$=0.28, p=0.60).

The minimal adequate GLM describing female response to male Lateral Display and Lead Swim included only the variable that described which of the males in a pair had the highest redscore. For female response to male Quiver, the magnitude of the difference in redscore remained in the model as well. The other variables, male yellowscore, blackscore and standard length did not significantly explain the remaining variation in female response.

The response of Kissenda females to male display behaviour did not differ between trials with Kissenda males and trials with Makobe males (all F<3.31, p>0.079). Yet, whereas the redscore differences in the two Makobe male pairs was larger than those in the Kissenda pairs, female preferences for male redscore were significantly weaker in trails with Makobe males (all F>5.84, p<0.022; Figure 6.2). Female preferences for male blackscore were slightly stronger in trials with Makobe males (4.10<F<4.30, 0.044<p<0.050). There were no differences in female response to male yellowscore or standard length (all F<3.93, p>0.054).

Comparison of Kissenda and Makobe experiments

In both series of intrapopulation trials, Makobe (Chapter 2) and Kissenda (this study), the total number of male displays and female positive responses were highly correlated ($F>41.6$, $p<<0.001$ for all displays, for both populations). Makobe males had higher display frequencies than Kissenda males ($F>5.22$, $p<0.025$ for all displays), but the relationship between male display and female response did not significantly differ between populations ($F<0.33$, $p>0.57$ for all displays). Preference consensus did not differ between populations either (Mann-Whitney-U test comparing the variance in female preference for 7 Makobe male pairs vs. 5 Kissenda male pairs: $Z<1.22$, $p>0.22$ for all displays). Likewise, the amplitude of female preference scores did not differ between populations ($F<3.36$, $p>0.071$ for all displays).

There was a significant difference between Kissenda and Makobe trials in the tendency of females to prefer the brighter red male in a pair (Figure 6.4). Pooling the data of both populations, there was a significant preference for redder males ($F>10.60$, $p<0.002$ for all displays), but this preference was weaker in Kissenda trials (significant for Lateral Display: estimate [interaction]=-7.88±3.73, $F_{1,77} = 4.62$, $p=0.035$ and for Lead Swim: estimate [interaction]=-20.68±8.21, $F_{1,68} =7.01$, $p=0.01$; not significant for Quiver: estimate [interaction]=-7.75±4.77, $F_{1,76} =2.71$, $p=0.10$). Conversely, there was an overall preference for males with high

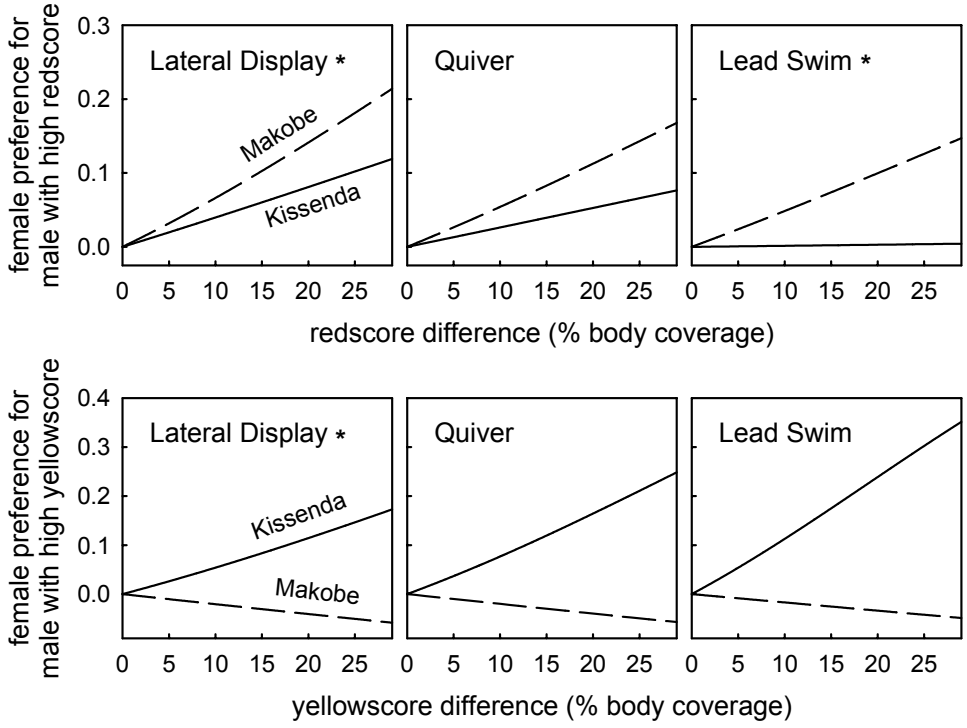


Figure 6.4 Preference curves (logit link functions) for Kissenda and Makobe females in the mate-choice experiments (intrapopulation trials only). Upper panel: female response to differences in male redscore; lower panel: female response to differences in male yellowscore. Curves are forced through the origin; asterisks denote significant differences in female response between populations.

yellowscores ($F>13.78$, $p<0.001$ for all displays), but this preference was stronger in Kissenda females (Lateral Display estimate [interaction]= 8.78±4.20, $F_{1,77}$= 4.44, p=0.038). The difference was not significant for Quiver and Lead Swim ($F<0.62$, $p>0.43$). There was no significant overall preference for high or low male blackscore ($F<2.11$, $p>0.15$), but whereas Makobe females tended to select against males with high blackscores, Kissenda females did not (interaction effects: $2.79<F<4.40$, $0.015<p<0.068$). Likewise, there was no overall preference for small or large males ($F<0.68$, $p>0.41$), but Kissenda females tended to prefer larger males whereas Makobe females did not (interaction effects: $2.05<F<4.28$, $0.017<p<0.14$).

Male coloration at Makobe and Kissenda Islands

Among wildcaught males, Makobe males had higher redscores than Kissenda males (n_1=28, n_2=17, MWU Z= -3.30, p=0.001; Figure 6.5). Yellowscore did not differ between populations (Z=-0.40, p=0.69; Figure 6.5). As a result, the yellow-to-red ratio was significantly higher in Kissenda males (median [range] Kissenda: 0.28 [0.01-5.49]; Makobe: 0.075 [0.01-0.73]; MWU Z=-2.44, p=0.015), giving the Kissenda males a more orange-ish appearance (Figure 6.1b). Moreover, the hue of male red coloration (dorsal hue) was significantly different between populations: Kissenda males had higher hue values, i.e. were more orange, than Makobe males (MWU Z=-2.46, p=0.014). These two measures are independent: the range of hues encountered in both populations was well within the chosen redscore criteria. Blackscore tended to be higher among Makobe males (Z=-1.71, p=0.087).

Kissenda males were significantly larger than Makobe males (standard length: Kissenda [n=17]: 90.6±1.6mm; Makobe [n=28]: 80.9±0.5mm; t=-5.64, $p<0.001$; weight: Kissenda: 19.1±0.7g; Makobe: 17.7±0.4g; t=-5.56, $p<0.001$), but had a lower condition factor than Makobe males (Kissenda: 2.57±0.06; Makobe: 2.77±0.05; t=2.54, p=0.015). Condition factor was negatively related to

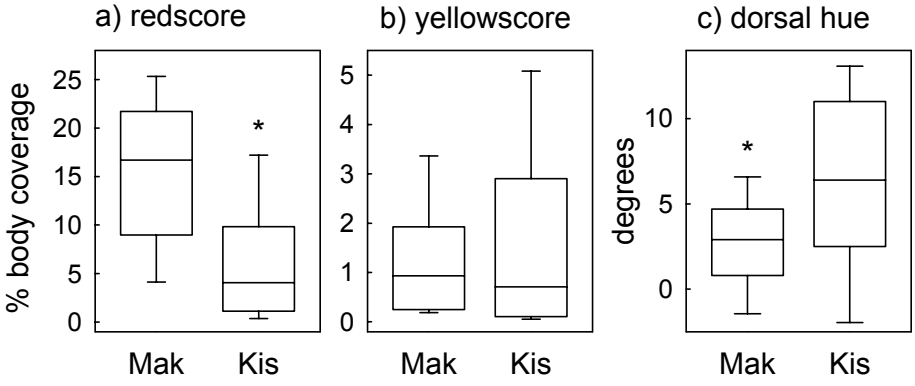


Figure 6.5 Nuptial coloration of wildcaught males from Makobe (Mak, n=28) and Kissenda (Kis, n=17). Boxes represent 75% confidence intervals intercepted by the median, error bars indicate 90% confidence intervals. a) Makobe males had significantly higher redscores than Kissenda males; b) yellowscore did not differ between populations. c) Makobe and Kissenda males differed significantly in dorsal hue: Kissenda males were more orange, whereas Makobe males were more red.

male redscore at Makobe (Pearson correlation, r=-0.44, p=0.02). At Kissenda Island this correlation was not significant (r=-0.37; p=0.14), but the relationship did not differ significantly between populations (GLM with condition factor and population as explanatory variables; effect of population: $F_{1,41}$<0.02, p>0.8). Yellowscore was not related to body condition in either population (Kissenda: r=-0.32, p=0.21, Makobe: r=-0.06, p=0.76).

Light transmission at Makobe and Kissenda Islands

The decline of light intensity with increasing depth was steeper at Kissenda Island than at Makobe (Figure 6.6): the overall light intensity in the Kissenda *P. nyererei* habitat (2-5 m depth) was less than 67% of that at Makobe (4-7 m). With increasing depth, the ambient light spectrum shifted towards longer wavelengths, resulting in an increasing 'orange-ratio' (the light intensity in the 550-700 range divided by the intensity in the 400-550 range [Endler & Houde 1995]; Figure 6.6cd). The orange-ratio increased faster at Kissenda than at Makobe, yielding 0.86 and 0.75 for the two *P. nyererei* habitats respectively. Thus, the *P. nyererei* habitat at Kissenda is darker and more red-shifted than it is at Makobe (Figure 6.6e). Given that at Kissenda, it was too dark for spectrophotometry beyond 3 m depth, the actual differences between the two *P. nyererei* habitats may be even larger.

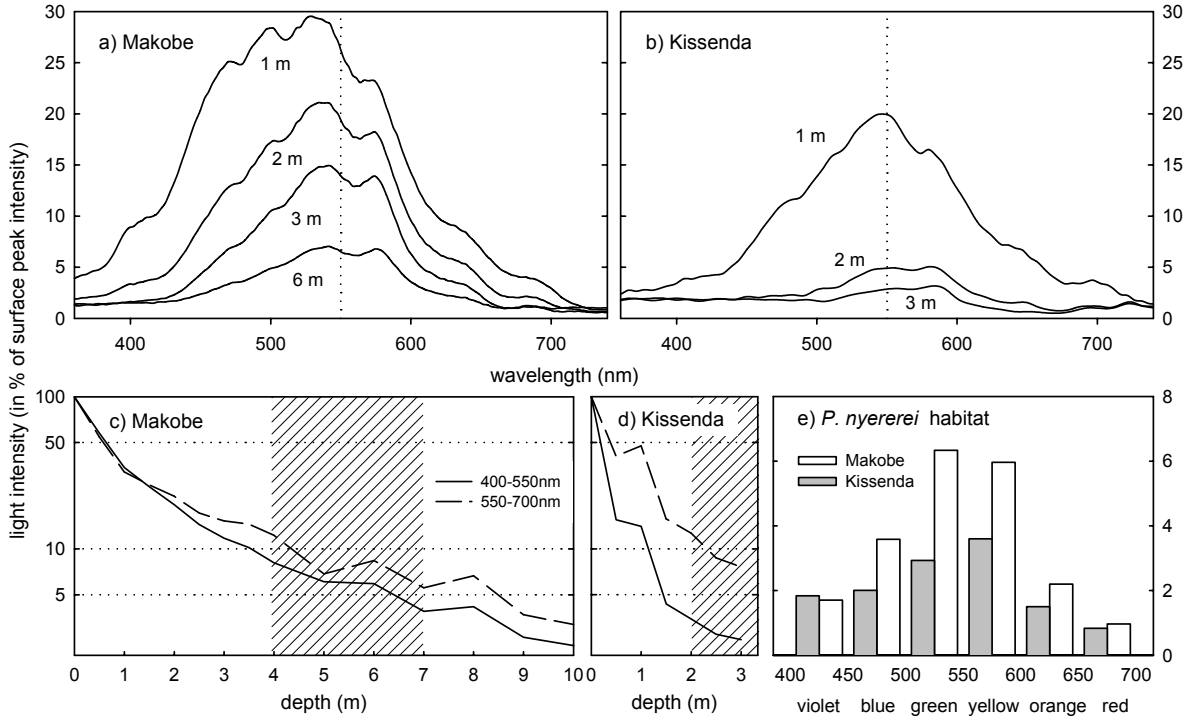


Figure 6.6 The photic habitat of *P. nyererei* at Makobe and Kissenda Islands. ab) Light spectra at different depths at Makobe and Kissenda. The dotted line indicates the 550 nm threshold, used for calculating the orange ratio. cd) Transmission of long (>550 nm, yellow-red) and short (<550, blue-green) wavelengths at both islands. Surface intensity is set at 100% for both wavelength classes. Axes are drawn to the same scale; shaded areas indicate *P. nyererei* habitat. e) Light intensity and spectral composition of the *P. nyererei* habitat at the two islands.

Discussion

Female *P. nyererei* mate choice behaviour differed significantly between Kissenda and Makobe populations. First, whereas females of both populations preferred redder males, this preference was significantly weaker in Kissenda than in Makobe females. Second, preferences were more strongly affected by male yellowscore in Kissenda than in Makobe females. Finally, Kissenda females preferred larger males but Makobe females did not. Females of the two populations did not differ in overall responsiveness to male courtship, overall choosiness or among-female consensus. Male coloration differed significantly between populations: Kissenda males had lower redscores and the hue of male red coloration was more orange-ish than in Makobe males.

These differences are probably associated with differences in light environment in the natural habitat of the fish. *P. nyererei* partly compensates for the low light transmission at Kissenda by inhabiting shallower water there than at Makobe. Also at other islands in south-eastern Lake Victoria, *P. nyererei* depth distribution varies with water transparency (including Makobe and Kissenda: $n=5$ islands, $r=0.96$, $p<0.01$; Figure 9.1). Despite this depth compensation however, the light environment of *P. nyererei* at Kissenda still differs from that at Makobe: it is darker and richer in long wavelengths. Therefore, the shift in male coloration from red towards yellow, both in body coverage and in hue, may be an adaptation to the ambient spectrum in this population: yellow and orange reflect more light than red and are better visible in a dark environment. Recent work indicates that also the visual system of *Pundamilia* has adapted to variation in the ambient light spectrum. Laboratory-bred F1 offspring from both *P. pundamilia* and *P. nyererei* from turbid water (Python Island, 10 km south-east of Kissenda, Secchi reading ~90 cm) were found to have more long-wavelength sensitive double cones than those from Makobe Island (Carleton et al. 2005).

Previous work (Chapter 3) has shown that the carotenoid composition of the red and yellow skin areas of male *P. nyererei* is very similar, and that the difference in hue is mainly due to differences in carotenoid content. This implies that the lower redscores and the more orange dorsal hue of Kissenda males correspond to a lower carotenoid content. Consequently, it could be argued that the population difference in male nuptial coloration is the result of a lower carotenoid availability at Kissenda Island, rather than an adaptive response to a different light environment. We think that this is unlikely for several reasons. First, the colour differences between Kissenda and Makobe males, as well as for other populations, persist in laboratory-raised F1 generations (Seehausen et al. 1997a; pers. obs.). Second, we have no indication that competition for food is more severe at Kissenda than at Makobe: *P. nyererei* population density (as well as total fish density) is lower (pers. obs.), and *P. nyererei* males are larger at Kissenda than at Makobe Island. Finally, the carotenoid-limitation mechanism yields predictions for female choice that are opposite to the findings presented here. As carotenoids become scarcer, carotenoid-based signals are more costly and thereby more informa-

tive (Andersson 1994; Grether 2000). Since *P. nyererei* red coloration is related to parasite load (Chapter 3), we would expect female preference for males with higher redscores to be stronger rather than weaker in carotenoid-limited environments. Parasite abundance and species composition may also differ between geographic locations. Likewise, variation in the strength and nature of predation pressure is likely to influence the expression of and (sexual) selection on male coloration (e.g. Endler 1983). Further studies must reveal how the relationships between *P. nyererei* male coloration, parasite infestation rates and female preferences vary with carotenoid availability, predation risk and water transparency.

In *P. nyererei* from Makobe Island, male coloration and behavioural courtship display are the most important choice criteria for females (Chapter 2). Therefore, the lower effectiveness of colour signalling in the turbid water of Kissenda Island could have selected for increased investment or elaboration of male courtship displays. We found the opposite of what would be expected according to such a mechanism: in the laboratory, Makobe males courted more frequently than Kissenda males. Similar differences exist between males from Makobe and males from the turbid waters of Python Island (Seehausen 1997). However, we found that Kissenda females preferred larger males whereas Makobe females did not, and that males were larger at Kissenda than at Makobe. Possibly, this reflects adaptation to an environment in which colour signalling is hampered.

We tested both populations in identical laboratory settings, with very clear water and an ambient light spectrum resembling daylight in shallow water. Our finding of significant population differences in female preferences under these conditions suggests that the observed correlation between male coloration and water transparency is not mediated by environmental variation alone. Rather, they indicate that female mating preferences have evolved in response to this variation. This implies that even when water transparency at Kissenda Island were to increase, male coloration would not evolve towards the condition at Makobe until female preferences would have adapted to the new signal transduction properties of the environment. This is the first evidence for intraspecific preference-trait coevolution in cichlid fish, confirming the earlier suggestion that the decrease in Lake Victoria water transparency affects both intraspecific sexual selection and interspecific mate choice (Seehausen et al. 1997a). The alternative hypothesis, that weaker female preferences are the result rather than cause of introgression of *P. pundamilia* genes, seems unlikely. This is because the variation in *Pundamilia* male nuptial coloration is clearly clustered in two distinct phenotypic classes, blue and red. Moreover, female preferences for these male types are probably fully divergent too. Species-assortative mating preferences have not been studied in this population, but females of the comparable population at Python Island show strong and consistent species-assortative mating preferences (Seehausen & Van Alphen 1998; Haesler & Seehausen 2005). These observations argue against strong admixture of species-specific genes for mating preferences and mating traits between *P. pundamilia* and *P. nyererei*.

Finally, the association between water transparency and *P. nyererei* depth distribution is likely to increase competition for food and space in shallow water. This mechanism may be of general importance in haplochromines, potentially causing additional extinctions through the reduction of ecological niche space. These threats underline the importance of measures to counteract the ongoing eutrophication of Lake Victoria.

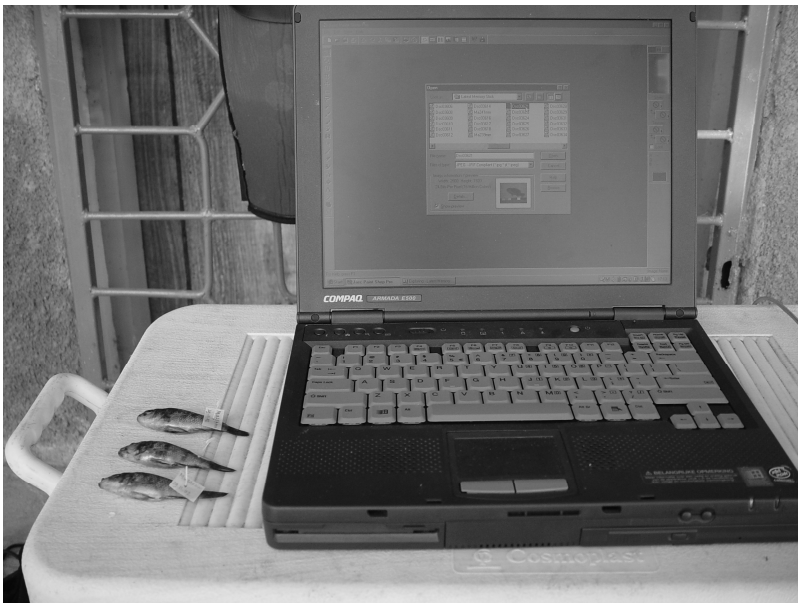
Acknowledgements

We thank the Tanzanian Commission for Science and Technology for research permission and the Tanzanian Fisheries Research Institute (Prof. Philip Bwathondi; Egid Katunzi) for hospitality and facilities. Mhoja Kayeba and Mohammed Haluna provided assistance in the field. We thank Hillary Mrosso, Peter Hiddinga, Roelof van der Kleij and Kees Hofker for their help with spectrophotometry, and Tom Van Dooren for helpful comments on the manuscript. The study was supported by the Netherlands Science Foundation (NWO-WOTRO 82-243) and a research grant from the Dobberke Foundation.

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

Martine E. Maan, Marcel P. Haesler, Ole Seehausen and Jacques J.M. van Alphen

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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: mean±se=223±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±2°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±se=359.5±3.3 days; n=310 offspring; in the laboratory, *N. omnicaeruleus* 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 Chi^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±se)	74.7±1.3	64.5±1.1	62.2±1.5	63.9±1.2	64.6±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±se)	2.1±0.1	2.2±0.1	2.2±0.2	2.0±0.1	2.0±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,

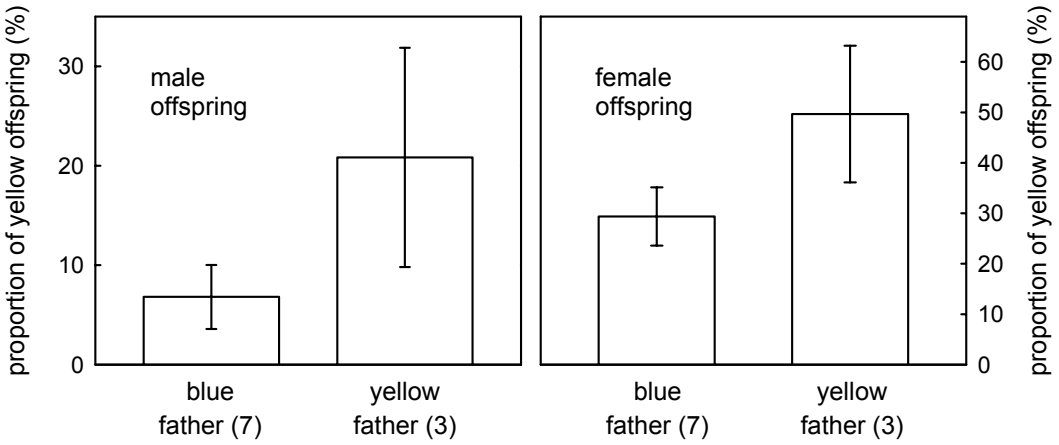

Figure 7.2 Results of the crossing experiment: the proportion of yellow individuals in the offspring of blue vs. yellow fathers. Left panel: male offspring; right panel: female offspring. Numbers in brackets indicate the number of clutches. Bars are means with standard errors.

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

mothers		fathers		male offspring			female offspring			clutch	sex ratio
no.	colour	no.	colour	Y	B	Y %	Y	GB	Y %	size	(% males)
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

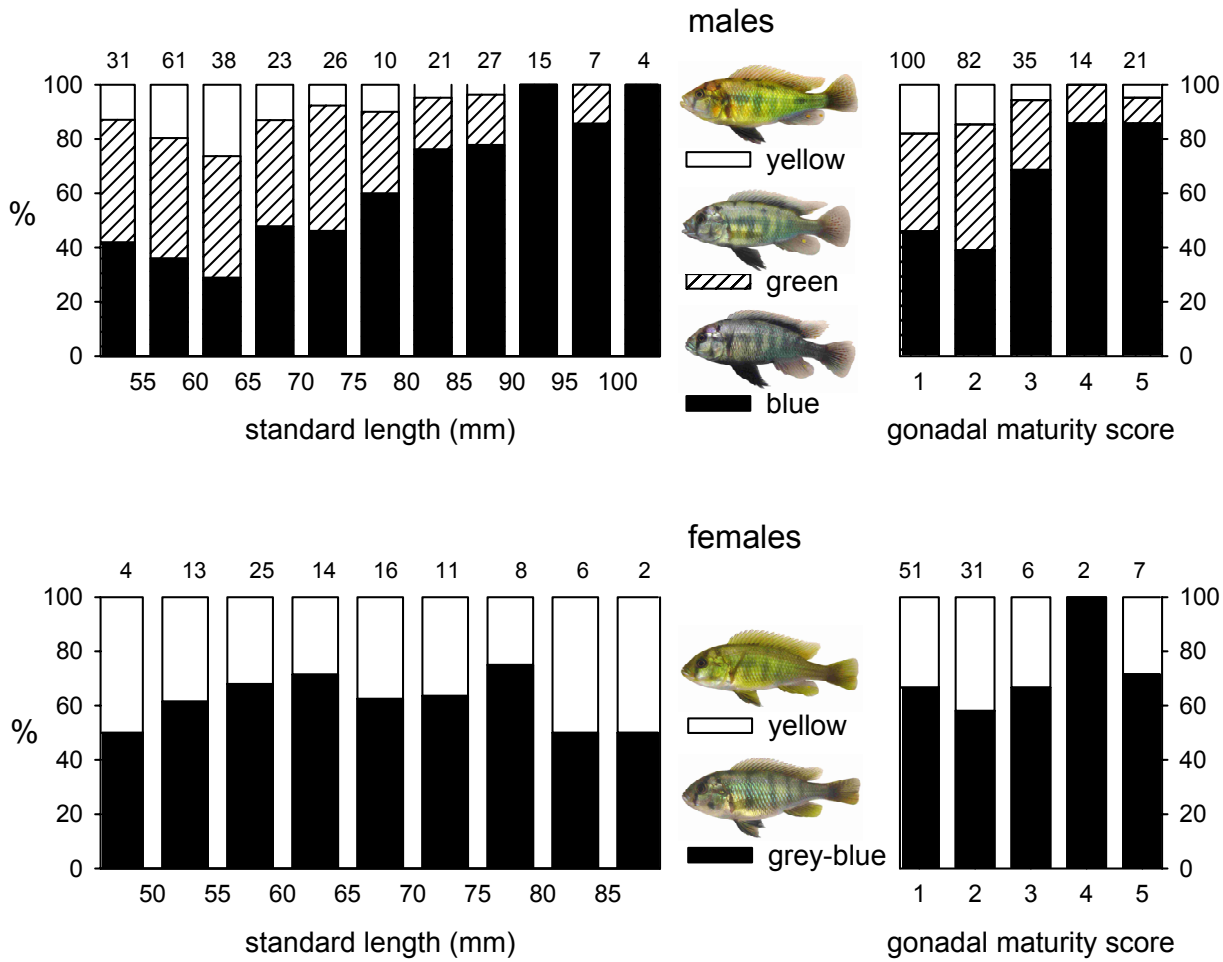

Figure 7.1 Frequency distributions for size (standard length) and gonadal maturity score of plain *N. omnicaeruleus* morphs at Makobe Island. Upper panel: males; lower panel: females. Numbers above the bars indicate sample sizes.

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±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.

Acknowledgements

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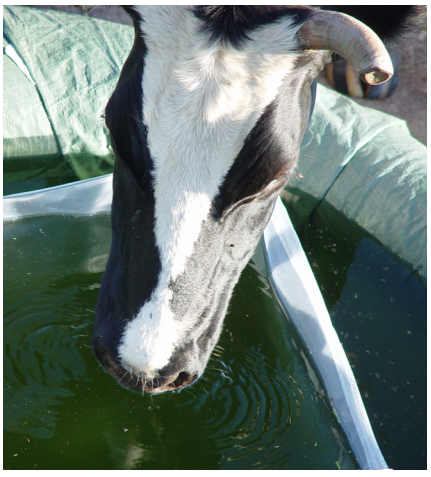
Chapter 8

'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.

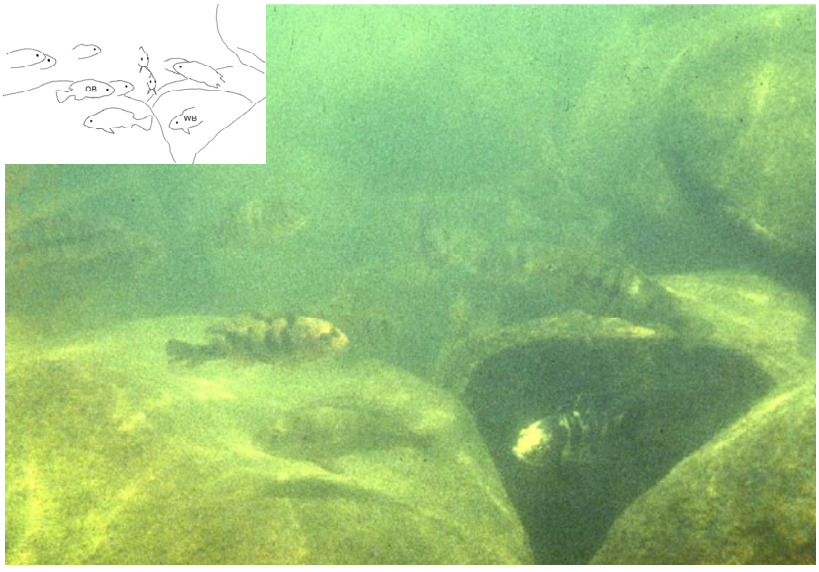

Figure 8.1
Neochromis omnicaeruleus in its natural habitat at Makobe Island: the algae-covered rocks at ~1.5 meters depth. The two blotched individuals, one orange-blotched and one white-blotched, are better discernable than the nine plain fish. Photo by OS.

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

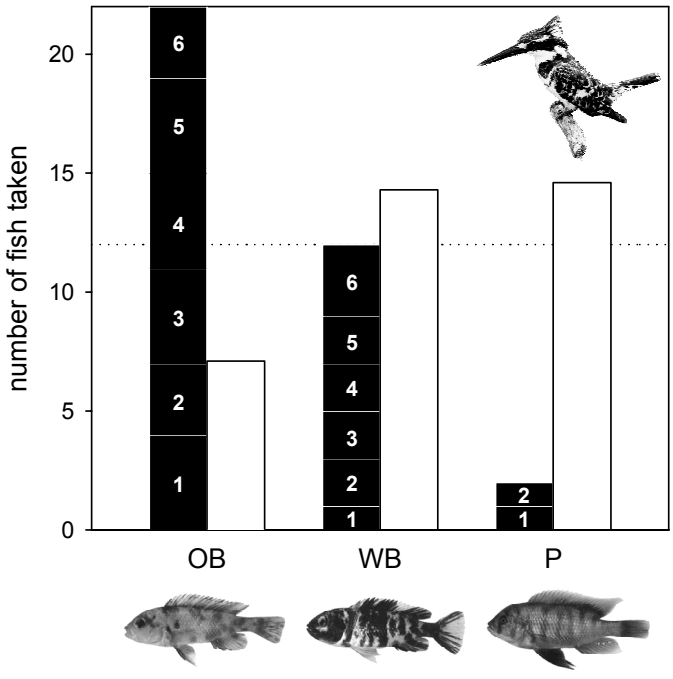

Figure 8.2 Predation frequencies for orange blotched (OB), white blotched (WB) and plain (P) *N. omnicaeruleus* in the experiment. For each morph, black bars represent the total number of fish taken in each of the six trials; trial numbers are indicated. Open bars indicate the expected numbers of fish 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).

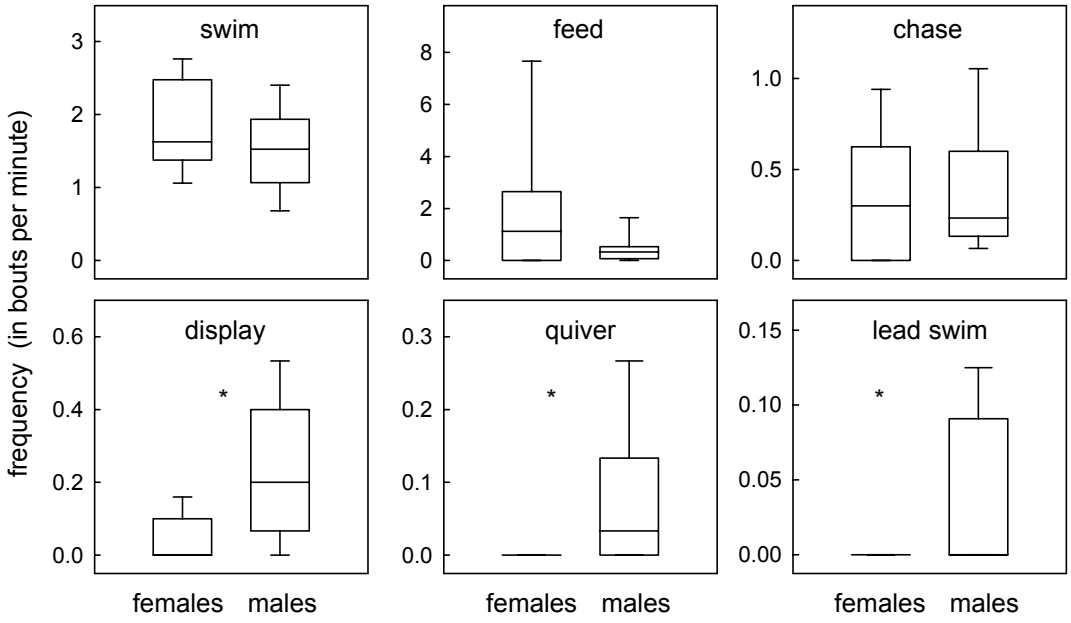

Figure 8.3 Behaviour of *N. omnicaeruleus* males (26) and females (9) at Makobe Island.

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,

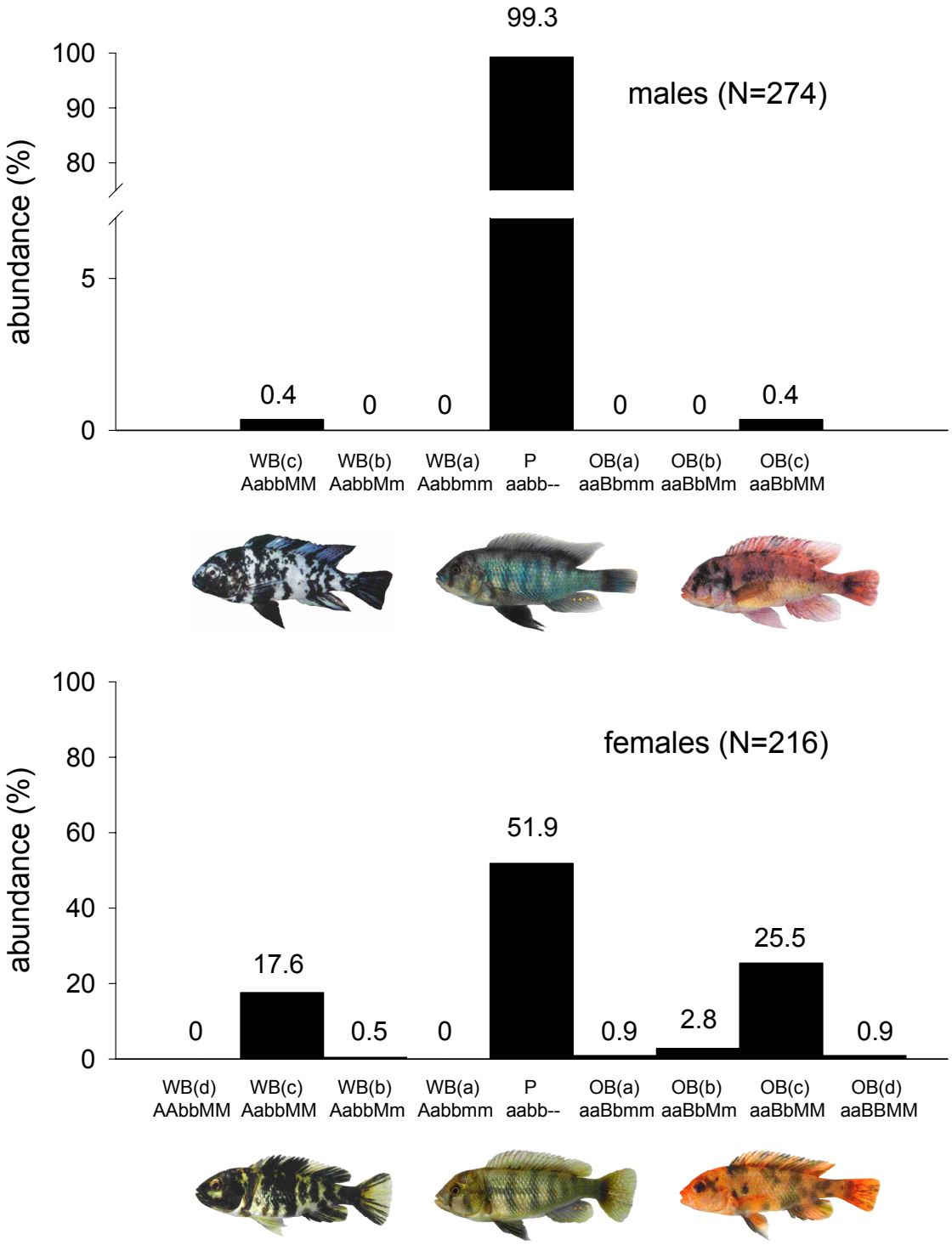

Figure 8.4. Abundances of *N. omnicaeruleus* phenotypes, with presumed genotypes, at Makobe Island. A,a: alleles at x-linked WB locus; B,b: alleles at x-linked OB locus; M,m: alleles at autosomal locus. For details on genotypes, see Methods text and Appendix 8.1.

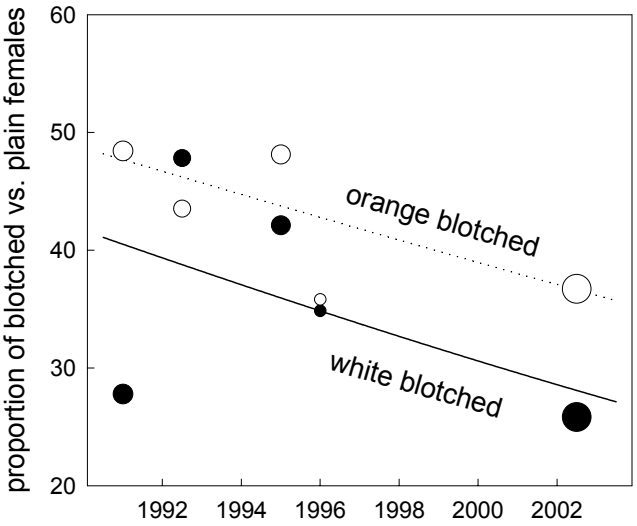

Figure 8.5. The decrease in the proportion of *N. omnicaeruleus* blotched females between 1991 and 2003 at Makobe Island. Symbol sizes indicate sample sizes: 1991:152 females, 1992-1993:129, 1995: 146, 1996: 90, 2002-2003: 216.

$n_{02\text{-}03}$=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).

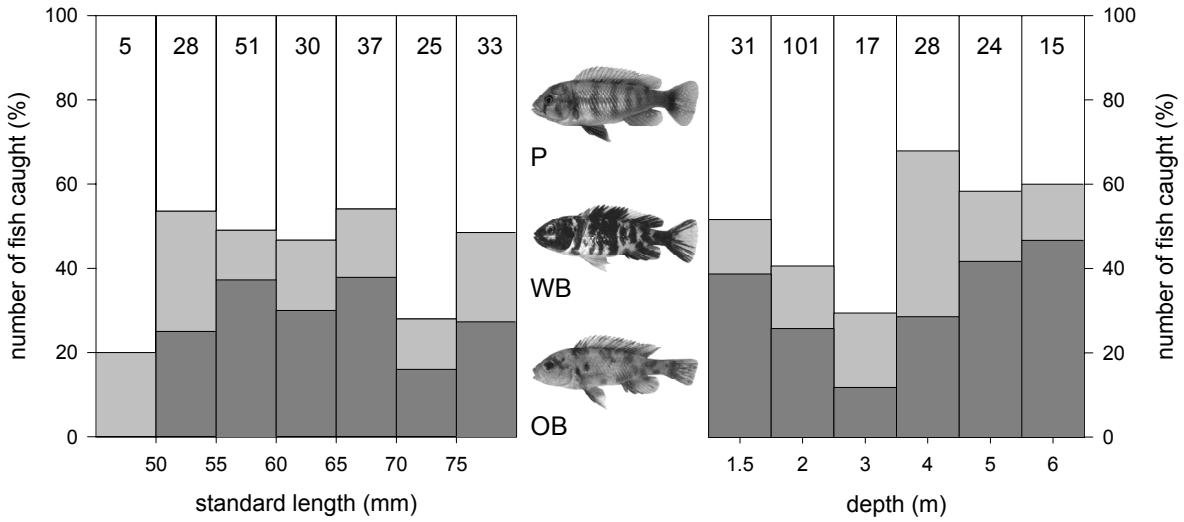

Figure 8.6. The numbers of females of the three *N. omnicaeruleus* colour morphs in different size (left panel) and depth (right panel) classes at Makobe Island. Numbers in the graphs indicate the sample size for each category.

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.

Acknowledgements

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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

Chapter 9

'The males sedulously court the females, and (…) take pains in displaying their beauty before them. Can it be believed that they would thus act to no purpose during their courtship? And this would be the case, unless the females exert some choice and select those males which please or excite them most. If the female exerts such choice, all the above facts on the ornamentation of the males become at once intelligible by the aid of sexual selection.'

Darwin, 1871 (on sexual selection in fishes)

Synthesis

In this final chapter, I summarise the main findings of the work presented in this thesis. I discuss their implications for our understanding of haplochromine speciation, and I briefly indicate how my results may affect the direction of future work.

Sexual selection within species as the basis for divergence?

Previous studies of haplochromine species diversity (Albertson et al. 1999; Knight 1999; Seehausen 1999) have provided support for the hypothesis that sexual selection on male nuptial coloration can drive haplochromine speciation. As a possible mechanism, Seehausen et al. (1997a) suggested that colour morphs or variants of the same species may become reproductively isolated due to divergent sexual selection. In this thesis, I have investigated several components of this mechanism in two genera of Lake Victoria haplochromines.

Firstly, the proposed mechanism entails that male nuptial coloration is subject not only to selection exerted by inter-specific mate choice, but also to intraspecific directional sexual selection (Lande 1981; West-Eberhard 1983; Boake 2002). In the closely related species pair *Pundamilia pundamilia* and *Pundamilia nyererei*, Seehausen & van Alphen (1998) had already demonstrated the former: females use male coloration as a cue in interspecific mate choice. As described in Chapter 2 of this thesis, I found that *P. nyererei* male nuptial coloration is subject to intraspecific directional sexual selection by female choice, thereby confirming the second prerequisite of the proposed mechanism for one of the two species. Future work should establish whether the same holds for *P. pundamilia*; the findings presented in Chapter 4 suggest that also in *P. pundamilia*, females may prefer to mate with the most conspicuously coloured males (see below).

Secondly, in the mechanism outlined above, the starting point for species divergence is provided by intraspecific colour variation that is genetically determined. In *Pundamilia*, heritability of male nuptial coloration has been established by laboratory breeding, both for the two reproductively isolated species, as well as for hybridising populations (Seehausen et al. 1997a; I. van der Sluijs pers. comm.; pers. obs.). In this thesis, I investigated another example of colour variation: the apparently continuous variation from yellow to blue in both sexes of *Neochromis omnicaeruleus*. In Chapter 7, I demonstrated that in this species, besides developmental and environmental factors, individual coloration is related to the colour of the parents. Hence, the studied population harbours genetic variation for coloration and/or the process of colour change. Since blue-red and blue-yellow colour polymorphisms and species pairs are common among Lake Victoria haplochromines (Seehausen et al. 1999c), these findings provide support for the hypothesis that species divergence could start from intraspecific colour variation. In the case of *N. omnicaeruleus*, the mechanism of ontogenetic colour change and its potential to respond to natural or sexual selection remains to be investigated. For

example, artificial selection in the laboratory may produce separate blue and yellow populations. Further, laboratory experiments could investigate the presence of colour-based mating preferences, and the physiological mechanisms and environmental factors that determine colour development.

The third major component of the speciation mechanism is a source of divergent selection on coloration and/or on colour preferences. In Chapter 5, I presented evidence to indicate that a heterogeneous light environment may exert divergent natural selection on visual properties and on male nuptial coloration. I found that females of *P. pundamilia* and *P. nyererei* differ in their behavioural responses to blue and red light, in a way that matches the difference in the hue of male nuptial coloration between the species. I proposed that the spectral depth gradient in the Lake Victoria waters entails that individuals with increased red-sensitivity, communicating by use of red signals, are at an advantage in deeper water, whereas individuals with increased blue-sensitivity, using blue signals, do better in shallow water. Several studies have identified genetic variation for colour perception in haplochromine cichlids (Carleton & Kocher 2001; Terai et al. 2002), including *P. pundamilia* and *P. nyererei* (Carleton et al. 2005). The experiment described in Chapter 5 is the first study that demonstrates interspecific differences in colour perception at the behavioural level, in a pair of very closely related species. Together with female preferences for conspicuously coloured males, as demonstrated for *P. nyererei* in Chapter 2, this divergent natural selection on visual properties provides a mechanism for population divergence.

It must be noted however that none of the studies mentioned above, including mine, have established a direct relationship between female colour perception (quantified as optomotor response thresholds, or inferred from opsin gene sequences or protein structure) and female mating preferences for male nuptial coloration. To assess the potential of divergent sensory drive as a mechanism of speciation, this relationship must be tested. This can be done by measuring both visual sensitivity and mating preferences in the same sample of individuals, preferably including interspecific hybrids. Artificial selection in the laboratory may provide additional support, when colour sensitivity and colour preference show a correlated response to selection. In guppies, selection lines with increased sensitivities to certain colours have been established (Endler et al. 2001), indicating that this approach may be fruitful also in cichlids. The considerably longer generation times in cichlids however will make that such experiments require several years.

The costs of bright colours: parasites and predators

Female preference for conspicuous males can evolve in many different ways, and several mechanisms may operate simultaneously (Andersson 1994). Females may simply mate with the first male they detect in order to minimise search costs. This strategy may be beneficial when potential mates are scarcely distributed or difficult to detect (e.g. in turbid water). As discussed in Chapter 5 (see above), female preferences for conspicuous colours can evolve as a by-product of the evolution of sen-

sory properties ('sensory bias', e.g. Ryan 1990; Endler 1992). When conspicuous signals are costly to produce or maintain, female choice for those signals may be driven by handicap selection: handicapping ornaments can only be afforded by individuals of sufficient quality (Zahavi 1975). Colours can be costly in several ways. They may require resources that would otherwise be available for other physiological processes, such as immune defence (Chapters 3 and 4). Further, bright colours may increase an individual's conspicuousness not only to choosy females, but also to predators (Chapter 8).

1) Parasite-mediated sexual selection

In both *P. pundamilia* and *P. nyererei*, I found that male nuptial coloration was related to infestation with macroparasites, with high colour scores correlating with low parasite loads (Chapters 3 and 4). In *P. nyererei*, the relationship between male red coloration and parasite resistance was recently confirmed in a laboratory study involving experimental infection of males of the same population (Dijkstra, Hekman, Schulz & Groothuis in prep.). Thus, in *P. nyererei*, the evidence suggests that carotenoids may mediate a trade-off between sexual signalling and immune defence, making male red coloration an honest signal of individual quality. For *P. pundamilia* however, the evidence is not as strong: my sample sizes were small and statistical power was limited. Moreover, the physiological mechanism underlying the relationship between blue coloration and immune defence remains unclear. Whereas in *P. nyererei* the trade-off between male nuptial coloration and parasite resistance may be mediated by carotenoids (Lozano 1994; Olson & Owens 1998), the blue coloration of *P. pundamilia* is carotenoid-independent. Further, as noted above, it is unknown whether female *P. pundamilia* exert directional sexual selection on the blue coloration of *P. pundamilia* males. Whereas Seehausen & van Alphen (1998) demonstrated that *P. pundamilia* females prefer conspecific males over *P. nyererei* males, and that they use male colour as a cue, we do not know which male traits determine intraspecific mate choice in *P. pundamilia*. Given the relationship with male parasite load (Chapter 4), we may expect *P. pundamilia* females to exert directional selection on male blue coloration. However, as is the case in *P. nyererei* (Chapter 2), *P. pundamilia* female preferences may be influenced by other male characteristics too, such as male size and display activity, territory size and quality, and other aspects of male coloration. In particular, the red coloration in the anal, caudal and dorsal fins of male *P. pundamilia* may affect female choice. This red coloration is not fully expressed until males are sexually mature, suggesting that it may play a role in sexual selection. Despite its carotenoid content however, the amount of red fin coloration was not related to parasite load. Possibly, it is intra- rather than intersexual selection that acts on these red fins: they may for example play a role in male-male competition for territories.

P. pundamilia and *P. nyererei* showed highly significant differences in the infestation rates of several parasite species, consistent with the ecological differences between the species. This indicates that *P. pundamilia* and *P. nyererei* differ in parasite exposure and/or resistance, and that they are subject to different selective re-

gimes in this respect. If trade-offs exist between resistance to the different parasite communities, niche differentiation could cause divergent selection due to differences in parasite exposure. Currently, there is no evidence for such trade-offs. However, given the habitat-dependent conspicuousness of male nuptial coloration, parasite-mediated sexual selection for conspicuous colours may contribute to the maintenance of reproductive isolation between the two species. This indicates that in a heterogeneous environment, good-genes sexual selection does not necessarily preclude species divergence by sexual selection. These findings are in line with recent theoretical studies which show that female preferences for locally adapted males may drive speciation (Edelaar et al. 2004; Reinhold 2004). Moreover, when fitness benefits accrued from female preferences are affected by both signal detectability and information content, they can be maximised in more than one preference-trait combination, especially in heterogeneous environments (Schluter & Price 1993).

Future work should address the heritability of parasite resistance. First, it should be tested whether female choice for bright red or bright blue males improves the parasite resistance of their offspring (e.g. Barber et al. 2001). Second, if parasite-mediated sexual selection is indeed divergent between the two species due to trade-offs in resistance, it follows that each species performs better in its own habitat than in the habitat of the other species, and that hybrids are at a disadvantage in both parental niches (Schluter 2001). Whereas experimental tests of this prediction may not be feasible, experimental infection in the laboratory could provide evidence for this hypothesis: trade-offs and hence divergent selection would be implicated when intraspecific hybrids have lower resistance than either parental species. Third, it may be worthwhile to study the relationships between male coloration and parasite load in populations with different levels of gene flow between *P. pundamilia* and *P. nyererei*: in turbid water populations, where the two species' niches overlap (see below), parasite-mediated divergent selection may be weak.

Finally, it must be noted that parasite resistance is only one of possibly many aspects of individual quality that females may assess during mate choice. My findings that female *P. nyererei* mate multiply, and that male coloration is not related to territory size or body size (Chapters 2 and 3), suggests that male fitness is a complex trait that cannot be assessed perfectly from just one or two characters. Therefore, to understand the evolutionary consequences of female selectivity, it would be important to further investigate the organisation of female mate choice decisions: how many males do females sample, is there variation among and within individual females, and do consecutive sires increase in assessed quality (e.g. Pitcher et al. 2003)?

2) Predation

Conspicuous colours and ornaments may increase the risk of predation. As a result, brightly coloured individuals that survive despite this disadvantage, will tend to possess compensatory survival abilities - they may be particularly good swim-

mers, or they manage to defend high-quality territories, or perhaps they are more vigilant than other individuals. According to the handicap principle, female choice evolves to provide the offspring with these qualities, yielding mating preferences for conspicuously ornamented males (e.g. Rosenthal et al. 2001; Godin & McDonough 2003).

In this thesis, I have not directly addressed this hypothesis, but my findings do support the idea that predator-mediated handicap selection may play a role in haplochromine colour evolution. In Chapter 8, I investigated whether differential predation with regard to colour and sex may contribute to the maintenance of a blotch colour polymorphism in *Neochromis omnicaeruleus*. I demonstrated experimentally that the blotched morphs may incur increased predation risk from visually hunting bird predators. Since sex-linked blotch polymorphisms occur in many cichlid species (Lande et al. 2001), differential predation may play a role in other species too. Moreover, I found that the orange-blotched fish in particular were attractive for the pied kingfishers, a result that may be extrapolated even further. For example, the bright red and yellow *P. nyererei* males may be very attractive to predators as well. Unfortunately, experiments using *P. nyererei* were unsuccessful: males do not develop their bright colours until they have reached a size that cannot be handled by pied kingfishers. To test the hypothesis that *P. nyererei* coloration is a handicap in terms of predation risk, other experimental designs are required. One could use aquarium-raised offspring from wildcaught fish, because these reach sexual maturity (including full expression of nuptial coloration) at a size that will be accepted by pied kingfishers. Alternatively, other predatory species can be used. Cormorants and otters for example are large enough to handle adult *P. nyererei* males. Moreover, in nature, these species are more important predators for *P. nyererei* than are pied kingfishers. Cormorants have been successfully used in predation experiments with sticklebacks (Vamosi 2002).

The results described in Chapter 8 present the first experimental evidence that the conspicuous colours of cichlid fish are costly in terms of increased predation. However, this Chapter aimed to test a more specific hypothesis: that the phenotype frequencies of *N. omnicaeruleus* in nature, which are inconsistent with expectations derived from laboratory crosses, could be explained by differential predation on blotched males (Seehausen et al. 1999b). Consistent with this hypothesis, I found that the blotched colour patterns (the orange-blotched pattern in particular) attracted more bird attacks, and that territorial defence and courtship behaviour would expose males more than females. However, the finding that blotched males were rare already as juveniles, suggests that either they are only rarely produced, or that they experience higher mortality at pre-reproductive age, rather than suffering from increased predation as sexually mature territorial males. Thus, whereas my study identifies differential predation as an important selection pressure, it does not suffice to explain the skewed distribution of blotched phenotypes among males and females in this species in nature. Probably, the underlying genetics of male rescue and blotch expression are more complicated than laboratory crosses suggested (Seehausen et al. 1999b). Additional ge-

netic factors may be identified in large-scale crossing schemes. Further, field observations on mating behaviour of different morphs and of both sexes will be required to distinguish genetic and environmental determinants of phenotype frequencies in nature.

Environmental constraints: the role of the ambient light spectrum

The low transparency of the Lake Victoria waters creates an underwater light environment that rapidly changes with depth. Moreover, water transparency varies considerably between different parts of the lake. This variation in ambient light spectrum is an important selection pressure in the evolution and maintenance of haplochromine colour diversity. In several Chapters of this thesis therefore, the role of the ambient light spectrum was explicitly addressed.

1) Intraspecific sexual selection along a transparency gradient

Seehausen et al. (1997a) showed that the rate of hybridisation between *P. pundamilia* and *P. nyererei* increases with decreasing water transparency, and that the saturation of *P. nyererei* red nuptial coloration is lower in turbid water populations. The authors proposed that turbid water precludes visual mate choice, resulting in relaxation of sexual selection on male coloration.

In Chapter 6 of this thesis, I investigated the mechanism underlying the correlation between male colour and water turbidity, comparing two populations from islands that differ considerably in water transparency. I showed that the two populations do not only differ in the amount and hue of male red coloration, but also in female mate choice behaviour, with females from turbid water being less choosy with regard to male red coloration. This indicates that male coloration and female choice co-evolve in response to variation in water transparency. The alternative hypothesis, that weaker female preferences are the result rather than cause of introgression of *P. pundamilia* genes, seems unlikely. This is because the variation in *Pundamilia* male nuptial coloration in this population is clearly clustered in two distinct phenotypic classes, blue and red. Moreover, female preferences for these male types are probably fully divergent too. Species-assortative mating preferences have not been studied in this population, but females of the comparable population of Python Island show strong and consistent species-assortative mating preferences (Seehausen & Van Alphen 1998; Haesler & Seehausen 2005). These observations argue against strong admixture of species-specific genes for mating preferences and mating traits between *P. pundamilia* and *P. nyererei*.

I also found that in the turbid waters of Kissenda Island, *P. nyererei* inhabits a shallower habitat than at Makobe Island where water transparency is relatively high. These population differences in depth distribution and in the hue of male coloration are consistent with general trends that exist across five populations of *P. nyererei* in the south-eastern part of the lake (Figure 9.1).

With increasing turbidity, *P. nyererei* moves to shallower habitats and the hue of male red coloration tends to become more orange. These changes are likely adaptations to the ambient light spectrum: in turbid water, light intensity decreases more rapidly with depth than in clear water, hampering visual detection of food, predators and potential mates. We now have evidence for three different mechanisms by which *P. nyererei* responds to this selection pressure. Firstly, *P. nyererei* moves to a more shallow habitat. This niche shift may have ecological consequences in terms of diet and competition with other species: increased water turbidity may reduce ecological niche space and increase competition for food and space in shallow habitats. The association between water transparency and depth distribution may apply more generally for Lake Victoria haplochromines, constituting a potential threat to local species diversity. A second adaptation was recently demonstrated by Carleton et al. (2005): in the turbid-water population from Python Island, both *P. nyererei* and *P. pundamilia* were found to have higher proportions of long wavelength-sensitive cones than those from the clear-water Makobe population. As such, the wavelength sensitivities of the fish correspond better

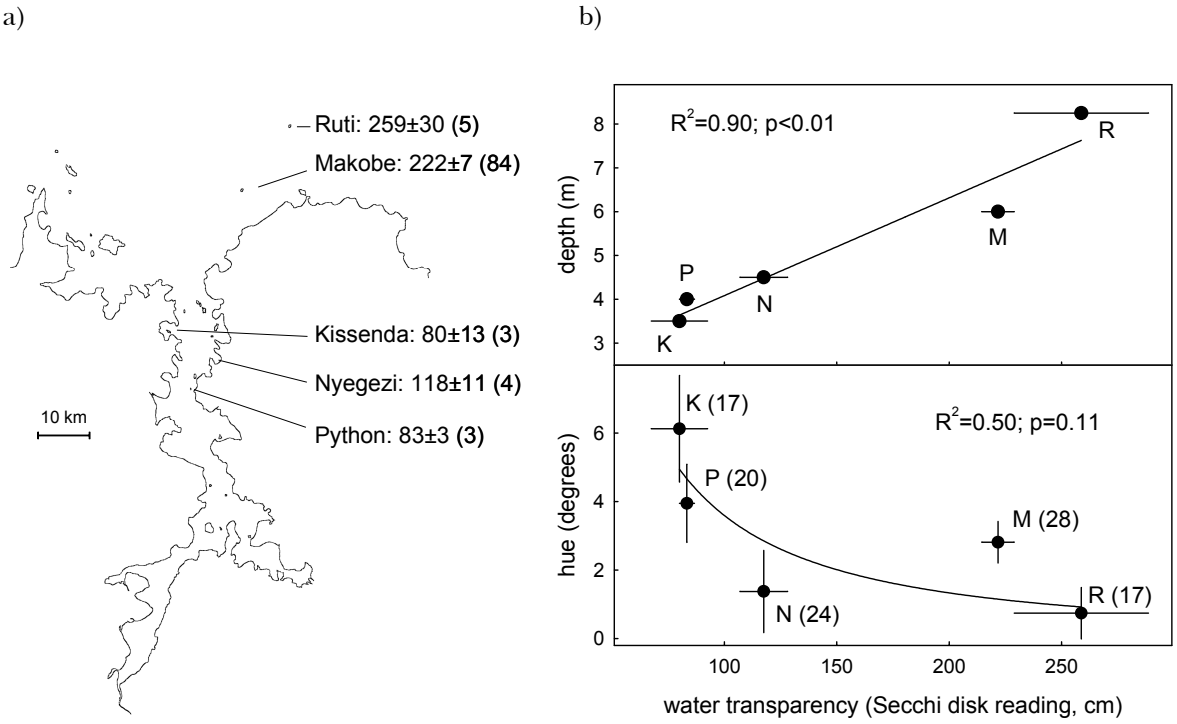

Figure 9.1 Five populations of *P. nyererei* at locations with different water transparencies. a) The Mwanza Gulf and western Speke Gulf with population locations and their water transparencies (mean and standard error of Secchi readings, with the number of measurements in brackets). b) Upper panel: the depth of maximum *P. nyererei* abundance, estimated from angling and gillnetting catches, and from underwater observations (for Ruti and Makobe Islands). Lower panel: the hue of male red coloration, measured in degrees on a standard colour wheel. Sample sizes are given in brackets. Locations: K=Kissenda Island; P: Python Island; N: Nyegezi; M: Makobe Island; R: Ruti Island. Data were collected in 2001-2003.

to the red-shifted ambient spectrum in more turbid water. Finally, I found that males from more turbid water had lower redscores and that females showed an associated decrease in selectivity with respect to this trait. Further work should establish whether female mate preferences in other populations follow the same tendency. Moreover, while the hue of male red coloration changed in a direction that is likely to enhance conspicuousness to females, my experiments did not test female preferences for male hue. Therefore, the hypothesis that a decrease in water transparency coincides with a shift in female preference for more orange-ish male coloration, remains to be tested.

As discussed in Chapter 6, the association between water transparency and sexual signalling also affects the information content of the male signal. Turbid waters may enable *P. nyererei* males to attract females with carotenoid-poor, relatively 'cheap' coloration that does not reliably predict male quality. This mechanism would provide an alternative explanation for a decrease in female selectivity with regard to male red coloration. Quantitative analysis of carotenoid availability and parasite infestation rates in different populations are required to investigate this hypothesis (e.g. Grether et al. 2005). Finally, in Chapter 4 I proposed that divergent sexual selection for parasite resistance contributes to the maintenance of reproductive isolation between *P. pundamilia* and *P. nyererei*. If, in turbid water, depth segregation is absent and male coloration does not reliably indicate quality, divergent selection may be weak. In these environments, reproductive isolation may not evolve. These ancestral *Pundamilia* populations are likely to resemble those present in initially clearer waters that have recently become turbid due to eutrophication. In these populations, secondary hybridisation may have caused mixture of *P. pundamilia* and *P. nyererei* genes into one hybrid gene pool. Molecular genetic tools may provide the means to distinguish between these alternatives for the *Pundamilia* populations in the southern Mwanza Gulf.

2) Water turbidity and visual predation

Visual predators are expected to spot conspicuous prey most easily in clear water (Cezilly 1992; Moyaho et al. 2004) and in the Mwanza Gulf, piscivorous birds tend to be more numerous on clear-water shores (Seehausen et al. 1997a; pers. obs.). Paradoxically, brightly coloured haplochromines, including the blotched phenotypes of several species, are most abundant in clear water (Seehausen & Bouton 1996; Chapter 6).

Conspicuousness is determined by a variety of factors, including the intensity and hue of the colour pattern, the background against which it is observed, the visual properties of the observer, and the intensity and spectral composition of the ambient light (Endler 1978). I cannot rule out the possibility that the blotched colour pattern of *N. omnicaeruleus* has a disruptive or camouflaging effect (Greenwood 1956; Snoeks 1994), especially in clear, shallow water, where sunlight produces irregular patterns of light and dark on the rocks. However, whereas this effect would explain the absence of blotched fish in turbid water, it would also lead

to decreasing numbers of blotched fish with increasing water depth. At Makobe Island, there was a significant trend in the opposite direction (Chapter 8).

As yet another explanation, the difference in conspicuousness between plain and blotched phenotypes may be smaller in clear than in turbid water. This is because the visibility of the plain fish may decrease faster with increasing turbidity than the visibility of the blotched fish. However, this hypothesis is inconsistent with the results of the predation experiment (Chapter 8): predation risk for blotched fish was much higher than for plain fish, although the experimental pond contained clear water and all fish were visible from above. This indicates that the difference in predation risk between plain and blotched morphs is not a direct function of water transparency.

Despite providing evidence for differential predation with regard to colour pattern, I conclude that my results suggest that the effects of sexual selection outweigh those of differential predation. Sexual selection on visual cues is likely to be stronger in clear water (Seehausen et al. 1997a; Järvenpää & Lindström 2004; Chapter 6). For example, the persistence of bright red *P. nyererei* at Makobe Island suggests that predation pressure is strongly opposed by sexual selection by female mate choice. Cormorants and otters were repeatedly seen in the *P. nyererei* habitat at Makobe, and we once observed a cormorant on the surface with a large *Pundamilia* 'pink anal' male in its beak. This species breeds sympatrically with *P. nyererei*. Nevertheless, among the territorial *P. nyererei* males that I observed there (n=28; Chapter 2), none disappeared during the five-week study period. The available evidence therefore suggests that, in providing an explanation for the evolution and persistence of haplochromine colour patterns along a turbidity gradient, the effects of visual predation are outweighed by the increased potential for visual mate choice in clear waters.

3) Species divergence along a spectral gradient

Besides geographical variation in turbidity between sites, the high organic content of the Lake Victoria waters creates habitat heterogeneity in light regime within sites: short wavelengths are selectively absorbed and scattered from the downwelling spectrum, resulting in a more red-shifted spectrum towards deeper water (De Beer 1989; Chapters 5 and 6). As a result, maximum conspicuousness of male nuptial coloration is achieved in different ways at different depths. Fish spectral sensitivities tend to match the ambient spectrum of the environment, in haplochromine cichlids (Van der Meer & Bowmaker 1995; Carleton & Kocher 2001; Carleton et al. 2005) as well as in other fish species (Loew & Lythgoe 1978; Bowmaker 1995; Cummings & Partridge 2001; Jokela et al. 2003). Therefore, female choice for conspicuous males may select for different male colours at different water depths. Together, these processes constitute divergent selection on male nuptial coloration and a possible mechanism for rapid speciation (Seehausen et al. 1997a; Carleton et al. 2005; Chapter 5).

The importance of inter- and intraspecific variation in visual properties for cichlid speciation is increasingly acknowledged (Sugawara et al. 2002; Terai et al.

2002; Spady et al. 2005). However, correspondence between differences in wavelength sensitivity, male nuptial coloration, female mating preferences and ambient light spectrum between sister species has now been established for only one species pair: *P. pundamilia* and *P. nyererei*. The generality of these findings is yet to be determined. In *N. omnicaeruleus* for example, I did not find any evidence for depth segregation between the blue and yellow colour morphs (Chapter 7). The distribution of differently coloured morphs or species is affected by several other selection pressures, such as predation, parasite exposure and dietary requirements. Moreover, recent studies indicate that male-male competition may be an important determinant of coexistence and spatial distribution of colour morphs and closely related species (Mikami et al. 2004; Seehausen & Schluter 2004; Dijkstra et al. 2005). Future work should establish the relative importance of these selection pressures and their interactions in speciation and species coexistence of haplochromine cichlids.

Conclusions

In this thesis, I have presented evidence for several selection pressures that co-determine colour evolution and population divergence in haplochromine cichlids. My results provide evidence that in *Pundamilia*, female choice for brightly coloured males (Chapter 2) may be driven by 'good genes' selection: brightly coloured males have lower parasite infestation rates (Chapters 3 and 4). Further, I have shown that species differences in female preferences for male colours coincide with species differences in visual sensitivities, indicating that mating preferences may evolve as a by-product of natural selection for increased visual performance (Chapter 5). Photic habitat heterogeneity provides the mechanism by which sexual selection through sensory drive becomes an agent of diversification. If the differences in parasite infestation rates between *P. pundamilia* and *P. nyererei* are due to differences in resistance rather than differences in exposure alone, and if there are trade-offs between the resistance to different parasites, female choice for resistant males could contribute further to the maintenance of reproductive isolation between these species (Chapter 4). By extrapolation (Chapter 8), I propose that *P. nyererei* nuptial coloration can be considered a 'handicap' in terms of predation pressure. Finally, variation in water transparency among *P. nyererei* populations has a profound influence on visual signalling between the sexes, with both female preferences and male colours changing with the signal propagation properties of the environment (Chapter 6).

In *Neochromis omnicaeruleus*, I showed that the colour variation that exists among the so-called 'plain' morph is determined by both genetic and environmental factors (Chapter 7). Pending further study, I suggest that this kind of intraspecific colour variability may provide a starting point for species divergence. In Chapter 8, I provided the first experimental evidence for differential predation on haplochromine colour patterns: blotched morphs of *N. omnicaeruleus* attract more predator attacks. Albeit insufficient to explain the skewed distribution of phenotypes in nature, my results indicate that differential predation with regard

to colour and possibly sex, is a selection pressure that must be taken into account in studies of haplochromine colour evolution.

My work has shown that the evolution of haplochromine colour patterns is subject to several simultaneous selection pressures. In particular, I have demonstrated that the interaction between sexual selection and habitat heterogeneity, in terms of photic environment and parasite exposure, may promote population divergence in male nuptial coloration and female preferences. In contrast, predation pressure and water turbidity may constrain the evolution and persistence of conspicuously coloured morphs and species.

Effective nature conservation requires insight in the biological processes that underlie the evolution and maintenance of biodiversity. Therefore, whereas my work primarily addressed fundamental questions about the mechanisms of haplochromine speciation, I hope that my results will eventually contribute to successful conservation of the Lake Victoria ecosystem.

Chapter 10

APPENDIX

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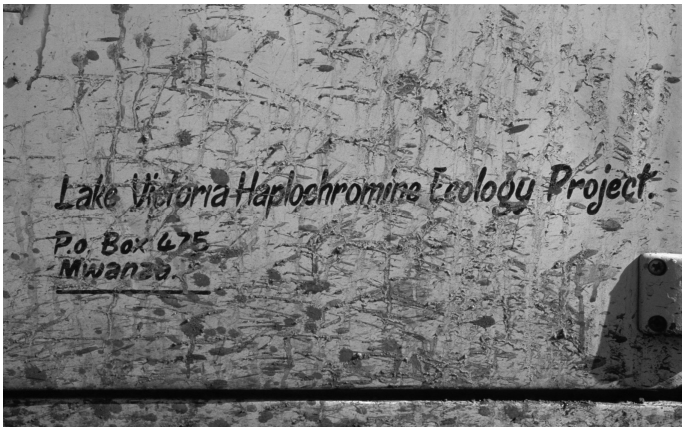
Lake Victoria Haplochromine Ecology Project.
P.O. Box 475
Mwanza.

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Voor de leesbaarheid heb ik de literatuurverwijzingen uit dit hoofdstuk weggelaten. Ze zijn te vinden in de hoofdstukken 1 en 9, die de basis vormen van deze samenvatting.

Soortsvorming

Soortsvorming (speciatie) is het ontstaan van nieuwe soorten: het splitsen van één soort in tweeën. Soortsvormingsprocessen nemen meestal duizenden of zelfs miljoenen jaren in beslag, veel te lang om binnen de spanne van een biologencarrière te kunnen volgen. Daarom is het onderzoek naar soortsvorming lang het domein geweest van taxonomen en systematici. Zij beschrijven de kenmerken en verwantschappen van (nieuwe) soorten, maar bestuderen niet het proces van soortsvorming zelf.

Evolutionair biologen onderzoeken de processen die soortsvorming veroorzaken. Deze processen vallen grofweg in twee categorieën: i) onwillekeurige, ongerichte processen, en ii) divergente[1] selectie. Klassieke studies van soortsvorming benadrukten de eerste: geografisch geïsoleerde populaties evolueren onafhankelijk van elkaar en wanneer ze elkaar na verloop van tijd opnieuw tegenkomen, zullen ze zich niet meer vermengen. Hun paringssystemen passen niet meer bij elkaar doordat toevallige veranderingen in fysiologie of gedrag zich hebben opgehoopt. Dan paren individuen van beide populaties niet meer met elkaar, of ze produceren onvruchtbare of niet levensvatbare nakomelingen. Zo blijven de populaties gescheiden, en zijn er dus twee nieuwe soorten ontstaan.

Soortsvorming kan ook het gevolg zijn van divergente selectie. In een heterogene omgeving kan, met of zonder geografische barrières, selectie ontstaan voor ecologische specialisatie en assortatieve partnerkeuze[2]. De uitwisseling van genetisch materiaal tussen subpopulaties ('gene flow'[3]) wordt dan zo gering, dat genetische verschillen behouden blijven en zelfs groter worden. Dit proces verklaart verschillende gevallen van 'adaptieve radiatie': de snelle evolutie van soortendiversiteit na kolonisatie van een nieuwe habitat, zoals een afgelegen eiland of een nieuw gevormd meer[4]. Bekende voorbeelden daarvan zijn de Oost-Afrikaanse cichlide vissen, fruitvliegen op Hawaï en de beroemde Darwinvinken van de Galápagos-eilanden.

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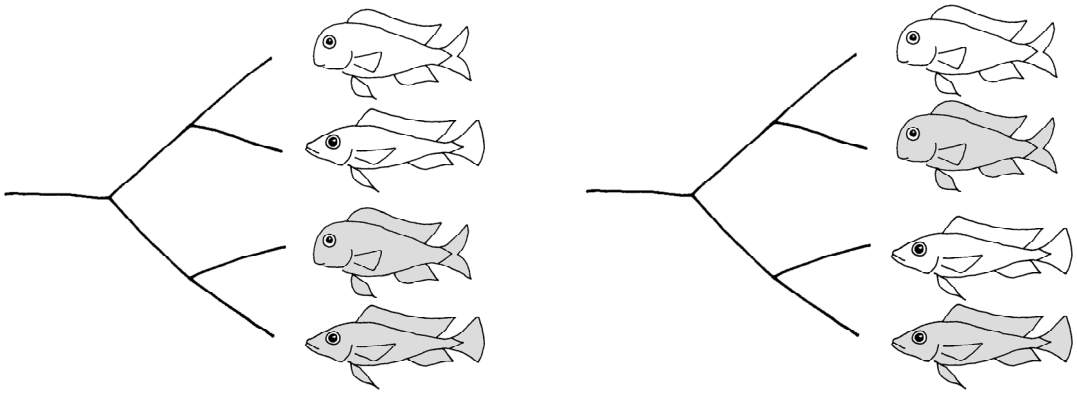
1 Divergent: in twee richtingen uiteenwijkend. Divergente selectie is selectie in twee richtingen.
2 Assortatief paren: paren met individuen die behoren tot dezelfde groep (populatie, soort, type).
3 Gene flow: het stromen van genetisch materiaal van de ene groep (populatie, soort, type) naar de andere door middel van voortplanting.
4 Adaptief: van het Latijnse *adaptare*, aanpassen.



Figuur 10.1 Verschillen tussen soorten kunnen worden gebruikt om af te leiden welke processen soortsvorming hebben veroorzaakt. Links: de meest nauwverwante soorten verschillen in morfologie, maar niet in secundaire geslachts kenmerken (kleur). Dat zou kunnen betekenen dat divergente seksuele selectie op kleur eerder heeft plaatsgevonden dan divergente natuurlijke selectie op morfologie en ecologie. Rechts lijkt soortsvorming anders te zijn verlopen: eerst divergente selectie op morfologie, daarna op kleur.

Divergente natuurlijke selectie

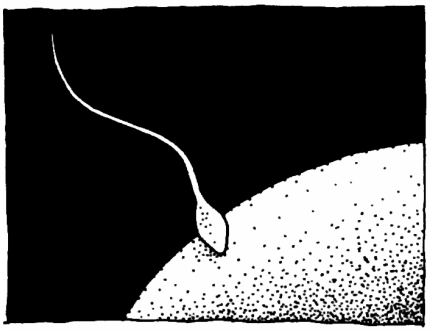
De theorie van ecologische soortsvorming door divergente natuurlijke selectie voorspelt dat jonge soorten vooral van elkaar verschillen in ecologische kenmerken zoals habitatgebruik, dieet en morfologie van het voedselopname-apparaat. De theorie voorspelt verder dat er in de niches van de beide nieuwe soorten selectie is tegen hybriden[5] met intermediaire fenotypen[6]. Als ecologische selectie de twee soorten uit elkaar heeft gedreven, zal elk van beide soorten goed zijn aangepast aan zijn eigen niche, en niet aan de niche van de andere soort. Hybriden tussen beide soorten zullen het in beide niches minder goed doen dan de 'pure' soorten en daardoor verdwijnen ze uiteindelijk uit de populatie. Beide voorspellingen komen uit voor de Darwin-vinken, een aanwijzing dat adaptieve radiatie in deze groep het gevolg is van divergente ecologische selectie.

In andere groepen daarentegen zijn ecologische verschillen tussen soorten helemaal niet zo groot, maar verschillen ze juist in secundaire geslachtskenmerken (Figuur 10.1). Dit zijn kenmerken die verschillen tussen de seksen, maar geen rechtstreekse functie hebben bij de voortplanting. Voorbeelden zijn de 'versieringen' of 'wapens' die bij veel soorten in mannetjes voorkomen, zoals de staart van de pauw of het gewei van herten. Deze kenmerken spelen een rol bij het verleiden van vrouwtjes en het imponeren van tegenstanders.

5 Hybride: nakomeling van een paring tussen individuen van twee verschillende soorten.
6 Fenotype: de verzameling uiterlijke kenmerken van een organisme (zoals vorm, kleur, gedrag). Vgl. 'genotype': de verzameling genetische (erfelijke) kenmerken van een organisme.

Divergente seksuele selectie

Kieskeurigheid bij het uitzoeken van een geschikte partner is een wijdverbreid fenomeen in het dierenrijk. Meestal zijn vrouwtjes selectiever dan mannetjes. Dat komt omdat vrouwtjes maar een beperkt aantal eitjes hebben, terwijl mannetjes enorme hoeveelheden zaadcellen kunnen produceren. Bovendien investeren vrouwtjes vaak meer tijd en energie in de nakomelingschap dan mannetjes: eieren zijn veel groter en kostbaarder dan sperma en vrouwtjes zorgen vaak langer of uitgebreider dan mannetjes voor de jongen (bij zoogdieren bijvoorbeeld in de vorm van zwangerschap en zogen). Deze veel voorkomende rolverdeling is soms aan het uiterlijk van de geslachten af te lezen: vrouwtjes zijn vaak onopvallend (om energie te sparen en geen roofdieren aan te trekken) maar de mannetjes zijn mooi versierd met felle kleuren, kuiven en waaierstaarten.



Figuur 10.2 Anisogamie: vrouwelijke geslachtscellen zijn veel groter dan mannelijke.

Wanneer nauwverwante soorten sterk verschillen in de kenmerken die betrokken zijn bij seksuele selectie, kan dat een aanwijzing zijn dat seksuele selectie een rol heeft gespeeld bij het proces van soortsvorming. Voorbeelden zijn Hawaiiaanse fruitvliegen, waarbij zustersoorten verschillen in chemische, akoestische en gedragsmatige aspecten van de balts, en de cichliden uit het Victoria- en Malawimeer, die grote diversiteit in kleur vertonen. Ook in andere taxa bestaan voorbeelden van subpopulaties of jonge soorten die verschillen in secundaire geslachtskenmerken en verder vrijwel identiek zijn. Deze observaties vormen de basis voor de hypothese dat soorten kunnen ontstaan door divergente seksuele selectie.

In theorie kan variatie in paringsvoorkeuren heel snel leiden tot soortsvorming. Daar staat tegenover dat theoretische modellen soms aannames maken die soortsvorming vergemakkelijken, maar die niet realistisch zijn. Ze veronderstellen bijvoorbeeld dat populaties veel genetische variatie bevatten voor partnervoorkeuren, terwijl het empirisch bewijs daarvoor mager is. Bovendien wordt vaak aangenomen dat kieskeurige vrouwtjes net zo veel nakomelingen krijgen als vrouwtjes die minder kieskeurig zijn of die een andere voorkeur hebben. Soms worden de kosten van kieskeurigheid meegewogen: een voorkeur voor een zeldzaam kenmerk kan leiden tot een lange en kostbare zoektocht. Maar er zijn maar weinig modellen die rekening houden met de voordelen van kieskeurigheid, terwijl daar talloze voorbeelden van zijn. Vaak kiezen vrouwtjes voor mannetjes die een veilig territorium, broedzorg, of genetische kwaliteit bieden. Het gevolg is dat variatie in voorkeur minder makkelijk ontstaan, zodat soortsvorming onwaarschijnlijk lijkt. Om vast te stellen of seksuele selectie tot soortsvorming kan leiden en hoe dat gebeurt, moet je daarom begrijpen hoe partnervoorkeuren tot stand komen en welke evolutionaire processen daaraan ten grondslag liggen.

Om deze vraag te beantwoorden, bestudeer ik in dit proefschrift de partnervoorkeuren, en de kenmerken waarop deze voorkeuren betrekking hebben, in de cichliden van het Victoriameer.

Oost-Afrikaanse cichliden

Met meer dan 2000 soorten zijn de Cichliden (Teleostei, Perciformes) de meest soortenrijke familie van gewervelde dieren. Ze komen voor in rivieren en meren in Afrika en Zuid- en Centraal-Amerika, met enkele soorten in Madagaskar en Azië. Meer dan driekwart van alle soorten cichliden leeft in de drie Grote Meren van Oost-Afrika: Tanganyika, Malawi en Victoria (Tabel 10.1, Figuur 10.3). In deze meren zijn in relatief korte tijd enkele honderden soorten ontstaan. Daarom zijn de cichliden een belangrijk modelsysteem voor het onderzoek naar adaptieve radiatie en explosieve soortsvorming.

Afgezien van enkele Tilapia-achtigen, behoren alle cichliden in het Malawi- en Victoriameer tot de 'haplochrominen', een monophyletische[7] groep van polygame, muilbroedende cichliden die wijd verspreid is over de rivieren en meren van Afrika. Net als het Tanganyikameer is het Malawimeer een diepe, smalle kloof met zeer helder water. Het Victoriameer daarentegen is veel minder helder en zeer ondiep. Het is ongeveer een half miljoen jaar geleden gevormd, maar verschillende malen opgedroogd, voor het laatst waarschijnlijk minder dan 15.000 jaar geleden. Toch leven in het meer minimaal 500 endemische[8] soorten haplochrominen.

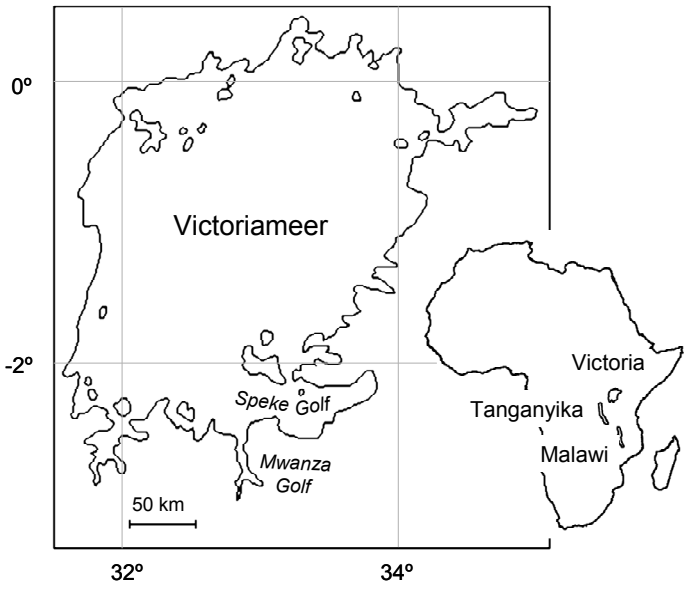
Tabel 10.1 Fysische en biologische kenmerken van de drie Grote Meren van Oost-Afrika.

	Tanganyika	Malawi	Victoria
ontstaan (in miljoenen jaren geleden)	9-12	4-5	0.25-0.75
maximum diepte (m)	1470	704	93
oppervlakte (km^2)	32.600	30.800	68.600
max. transparantie (Secchi schijf, m)*	22	17	8.2
min. en max. schattingen van het aantal soorten endemische[8] cichliden	162-250	451-1000	447-1000

*Een Secchi schijf is een zwart-wit beschilderde schijf die men vanaf het wateroppervlak in het water laat zakken, totdat de schijf niet meer te zien is. Deze diepte is een maat voor de helderheid van het water. Maximum transparantie in mijn studiegebied (in 2000-2003): Mwanza Golf 1.6 m; Speke Golf 4.0 m.

7 Monophyletisch: afstammend van één gemeenschappelijke voorouder. Endemische soorten komen uitsluitend in het betreffende gebied voor.

8 Endemische soorten komen uitsluitend in het betreffende gebied voor.



Figuur 10.3 De drie grote meren van Oost-Afrika.

Haplochromine soortsvorming

De evolutionaire geschiedenis van de Victoriacichliden is het onderwerp van voortdurende revisie en discussie. Er is wel overeenstemming over de uitzonderlijke snelheid van haplochromine soortsvorming in de Oost-Afrikaanse meren. De veranderlijke anatomie van cichliden, in het bijzonder hun voedselopname-apparaat, heeft waarschijnlijk bijgedragen aan hun evolutionaire succes: functionele flexibiliteit vergemakkelijkt snelle aanpassing aan verschillende ecologische niches. Maar zoals eerder genoemd verschillen nauwverwante haplochrominen vaak maar weinig in ecologie en morfologie. In plaats daarvan verschillen zustersoorten vaak in kleur.

De broedzorg bij haplochromine cichliden is zeer ongelijk verdeeld over beide geslachten: vrouwtjes nemen de volledige broedzorg op zich. Haplochromine mannetjes verdedigen een territorium en proberen vrouwtjes te lokken met baltsgedrag. Vrouwtjes beantwoorden deze balts door het mannetje te volgen naar het hart van zijn territorium en uiteindelijk met hem te paren. Vervolgens verlaat het vrouwtje het territorium met de eieren in de bek ('muilbroeden'). De investering van het vrouwtje in de jongen is aanzienlijk: allereerst de kostbare productie van de eieren zelf, die net als bij andere muilbroeders relatief groot en voedselrijk zijn. Vervolgens houdt ze de eieren twee tot drie weken in de bek. In deze periode kan ze niet eten; ze moet dus in goede conditie zijn om dat vol te houden. Tenslotte volgt een periode waarin ze de jongen bewaakt en zelfs weer terug in de bek neemt bij dreigend gevaar. Daardoor kunnen vrouwtjes tijdens hun leven maar een beperkt aantal nestjes produceren. Mannetjes investeren helemaal niets in de broedzorg. Hun enige bijdrage aan de jongen is hun genetisch materiaal. Deze asymmetrie leidt tot sterke seksuele selectie: vrouwtjes zijn kieskeurig bij het uitzoeken van een mannetje.

De combinatie van deze beide kenmerken van haplochrominen - sterke seksuele selectie en grote diversiteit aan kleuren - vormen de basis voor de hypothese dat seksuele selectie op kleurpatronen een rol speelt bij haplochromine soortsvorming.

De kleuren van haplochromine cichliden

Er zijn twee typen kleurvariatie die veel voorkomen in haplochromine cichliden, in zowel het Malawi- als het Victoriameer. Het meest algemeen in beide meren zijn blauw-rode of blauw-gele varianten. Voorbeelden zijn *Pundamilia pundamilia* (blauw) en *Pundamilia nyererei* (rood) in het Victoriameer (Figuur 5.1, pagina 81) en soorten in de genera *Pseudotropheus* en *Aulonocara* in het Malawimeer. Daarnaast komt in beide meren in verschillende soorten het zogeheten 'blotch polymorfisme' voor. Blotched individuen hebben zwarte vlekken op een witte of oranje achtergrond; homozygote[9] vissen zijn helemaal wit, zwart of oranje. In het soortsvormings-onderzoek zijn vooral de blotch polymorfismen van *Neochromis omnicaeruleus* (Victoriameer; Figuur 8.4, pagina 128) en *Maylandia zebra* (Malawimeer) als modelsysteem gebruikt.

Experimenteel onderzoek heeft aangetoond dat beide vormen van kleurvariatie een rol spelen in partnerkeuze, zowel binnen als tussen soorten. Dat bevestigt een belangrijke aanname van de hypothese dat divergente seksuele selectie op kleurpatronen bijdraagt aan soortsvorming in deze vissen, namelijk dat verschillen in kleur en kleurvoorkeur ervoor zorgen dat soorten niet met elkaar paren, zelfs wanneer ze in hetzelfde gebied voorkomen en in principe levensvatbare en vruchtbare hybriden zouden kunnen produceren. Bovendien lijken de kleurvariaties *binnen* soorten op de kleurvariaties die *tussen* soorten bestaan: een aanwijzing dat de laatste uit de eerste zijn ontstaan. In dit proefschrift heb ik deze hypothese verder onderzocht. Als vertegenwoordigers van de typische haplochromine kleurvariaties heb ik de drie eerder genoemde soorten uit het Victoriameer gekozen als modelsysteem: *Pundamilia nyererei*, *Pundamilia pundamilia* en *Neochromis omnicaeruleus*. In deze soorten heb ik de evolutionaire mechanismen onderzocht die verantwoordelijk zijn voor het ontstaan van (variatie in) kleurvoorkeuren.

Seksuele selectie in haplochromine cichliden

Er zijn talloze biologische theorieën die een verklaring bieden voor het ontstaan van partnervoorkeuren voor bepaalde eigenschappen. Kieskeurigheid hangt samen met datgene wat de partner te bieden heeft: een stevig nest, trouwe broedzorg, of 'goede genen'. De evolutionaire mechanismen die partnerkeuze bepalen, verschillen onderling sterk in de mate waarin ze variatie in voorkeur bevorderen of juist beperken. Daarom is het in de context van soortsvorming belangrijk om vast te stellen welk mechanisme actief is. Hieronder volgt een korte opsomming van de belangrijkste hypothesen, en hun relevantie voor haplochromine cichliden.

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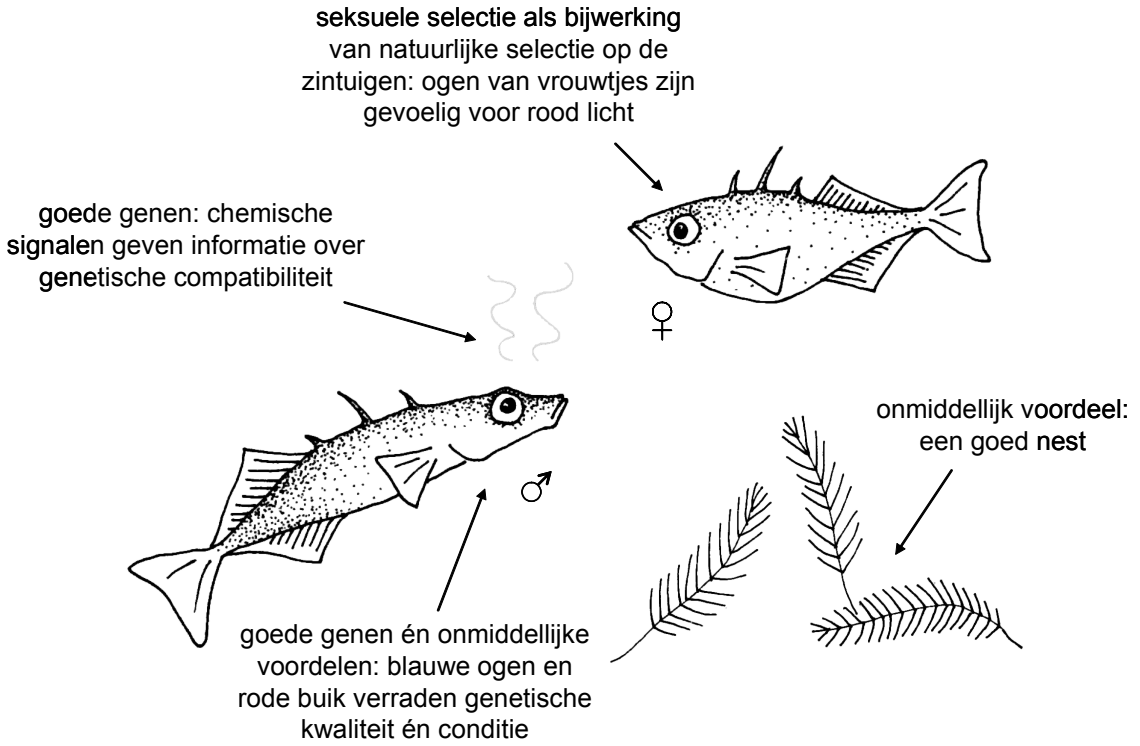
9 Homozygoot: met van beide ouders dezelfde versie van een gen. Vgl. heterozygoot: met twee verschillende versies.

1. Seksuele selectie voor onmiddellijke voordelen: het gekozen geslacht verstrekt broedzorg, voedsel, nestgelegenheid of andere voordelen. Een voorbeeld is de driedoornige stekelbaars, waarbij mannetjes een nest bouwen en de jongen van verschillende vrouwtjes grootbrengen. Vrouwtjes beoordelen de vaderlijke kwaliteiten van mannetjes door op hun felrode buik en blauwe ogen te letten. In verschillende soorten vogels (meeuwen, sterns) en insecten (krekels, vlinders) bieden mannetjes voedsel aan als onderdeel van het baltsritueel. In haplochromine cichliden zijn dergelijke onmiddellijke voordelen waarschijnlijk niet zo belangrijk, omdat mannetjes geen rechtstreekse bijdrage leveren aan de broedzorg of aan de conditie van het vrouwtje (en vrouwtjes zijn meestal het kieskeurige geslacht).

2. Seksuele selectie voor genetische kwaliteit: het gekozen geslacht levert 'goede genen' die de overleving of het voortplantingssucces (samen *fitness* genoemd) van de jongen vergroten. Zo ontstaat bijvoorbeeld een voorkeur voor oudere partners, omdat deze kennelijk goede 'overlevingsgenen' hebben. Een speciale vorm is 'handicap selectie'. Volgens dit mechanisme worden bepaalde kenmerken geprefereerd, juist omdat ze de overleving van de drager bedreigen – doordat ze kostbaar zijn om te produceren, opvallend zijn voor predatoren, of onhandig om mee te slepen. Vanwege deze kosten kunnen alleen individuen van uitzonderlijke kwaliteit zich dergelijke kenmerken veroorloven en dat is de reden dat er voorkeur bestaat voor deze kenmerken. Een bekend voorbeeld is de staart van de pauw, maar ook de felle rode kleur van de stekelbaars. 'Goede genen' kunnen ook ten goede komen aan het voortplantingssucces van de nakomelingen: als aantrekkelijke kenmerken erfelijk zijn, zullen de nakomelingen van een individu met deze kenmerken, zelf ook aantrekkelijk zijn. Dit soort selectie voor 'sexy sons' kan leiden tot een evolutionair sneeuwbaleffect, waarbij steeds grotere of spectaculairdere versieringen ontstaan – totdat natuurlijke selectie ingrijpt en de overlevingskans van superaantrekkelijke individuen zo laag wordt dat het kenmerk weer verdwijnt of de soort zelfs uitsterft. Ten slotte kunnen kieskeurige individuen een voorkeur hebben voor genetische kenmerken die goed passen bij hun eigen genetische achtergrond. Een bekend voorbeeld hiervan is selectie voor genen die belangrijk zijn voor het afweersysteem. In verschillende soorten zoogdieren en vissen kiezen vrouwtjes voor mannetjes die een zodanige verzameling van die genen hebben, dat ze samen met die van het vrouwtje de ideale combinatie voor de nakomelingen opleveren. Een belangrijk kenmerk van deze vorm van seksuele selectie is dat de 'ideale partner' voor iedereen anders is. Verder draagt selectie voor compatibiliteit vaak bij aan het verminderen van inteelt.

   In haplochromine cichliden zou selectie voor 'goede genen' een belangrijke rol kunnen spelen bij het ontstaan van kieskeurigheid. Daarom komt dit mechanisme expliciet aan de orde in dit proefschrift (Hoofdstukken 3,4, en 8).

3. Seksuele selectie als bijwerking van natuurlijke selectie op de zintuigen. Door natuurlijke selectie zijn zintuigen zodanig geëvolueerd dat ze een organisme in staat stellen om efficiënt de omgeving waar te nemen, voedsel te zoeken en predatoren op te merken. Dergelijke selectie leidt ertoe dat een organisme voor sommige signalen ontvankelijker is dan voor andere. Die gevoeligheden kunnen partnerkeuze beïnvloeden ('sensory drive'), en de gekozen sekse kan die gevoeligheid uitbuiten ('sensory exploitation'). Het meest beroemde voorbeeld van dit mechanisme zijn kikkermannetjes die kwaken op precies die toonhoogte waarvoor vrouwtjes het meest gevoelig zijn. In Hoofdstuk 5 van dit proefschrift onderzoek ik dit mechanisme voor de rode en blauwe *Pundamilia*'s.



Figuur 10.4 De driedoornige stekelbaars is een belangrijk modelsysteem in de gedragsbiologie en seksuele selectie is in deze soort uitgebreid onderzocht. Verschillende mechanismen zijn tegelijk actief.

Dit proefschrift: de hoofdstukken

Steeds meer studies ondersteunen het verband tussen kleurendiversiteit, seksuele selectie en soortenrijkdom van haplochromine cichliden. Toch is grotendeels onduidelijk hoe variatie in kleur en kleurvoorkeur eigenlijk ontstaat, en welke mechanismen van seksuele selectie dat proces hebben beïnvloed. Met dit proefschrift heb ik geprobeerd om op die vraag het begin van een antwoord te vinden.

Hoofdstukken 2 tot en met 6: blauw en rood. Divergentie van *Pundamilia pundamilia* en *Pundamilia nyererei*

Pundamilia pundamilia en *Pundamilia nyererei*[10] zijn twee nauw verwante haplochrominen die voornamelijk verschillen in de kleur van de mannetjes: grijsblauw respectievelijk fel geel met rood (zie Figuur 5.1 op pagina 81). De vrouwtjes van beide soorten zijn onopvallend bruingrijs en daardoor moeilijk van elkaar te onderscheiden. De geografische verspreiding van *P. nyererei* valt geheel binnen die van *P. pundamilia*, maar *P. nyererei* leeft en broedt in dieper water. De soorten hybridiseren in troebel water, maar niet in helder water. Vrouwtjes hebben een voorkeur voor de mannetjes van hun eigen soort, en de kleur van de mannetjes geeft daarbij de doorslag. Ik onderzoek de hypothese dat seksuele selectie voor verschillende kleuren de beide soorten uit elkaar gedreven heeft. Deze hypothese voorspelt dat de kenmerken die bepalend zijn voor reproductieve isolatie[11] *tussen* soorten, ook belangrijk zijn voor partnerkeuze *binnen* soorten. Met andere woorden: als de kieskeurigheid van vrouwtjes, voor blauwe of juist rode mannetjes, de soorten uit elkaar gedreven heeft, zullen vrouwtjes ook bij het kiezen tussen soorteigen mannetjes, dit kenmerk (kleur) gebruiken om het beste mannetje te selecteren. In **Hoofdstuk 2** heb ik deze voorspelling getoetst voor de soort met de rode mannetjes, *P. nyererei*. Ik heb de kieskeurigheid van vrouwtjes op drie verschillende manieren onderzocht: in een partnerkeuze-experiment in het aquarium, tijdens onderwater-observaties van territoriale mannetjes in het veld, en in vijvers waarin de vissen daadwerkelijk konden paren. Ik heb gevonden dat de rode kleur van de mannetjes ook voor de partnerkeuze *binnen* de soort het belangrijkste criterium is: vrouwtjes hadden een voorkeur voor de meest felrode mannetjes. Verder vonden vrouwtjes mannetjes met grote territoria aantrekkelijk. Ten slotte wezen de resultaten van het vijver-experiment erop dat mannetjes zonder territorium helemaal niet paren, en dat vrouwtjes de eieren van één nestje laten bevruchten door meerdere vaders.

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10 *Punda milia* betekent 'gestreept paard', ofwel zebra, in het KiSwahili. *P. nyererei* is genoemd naar de eerste president van het onafhankelijke Tanzania, Julius Nyerere. Zie Witte-Maas & Witte (1985) en Seehausen et al. (1998a).

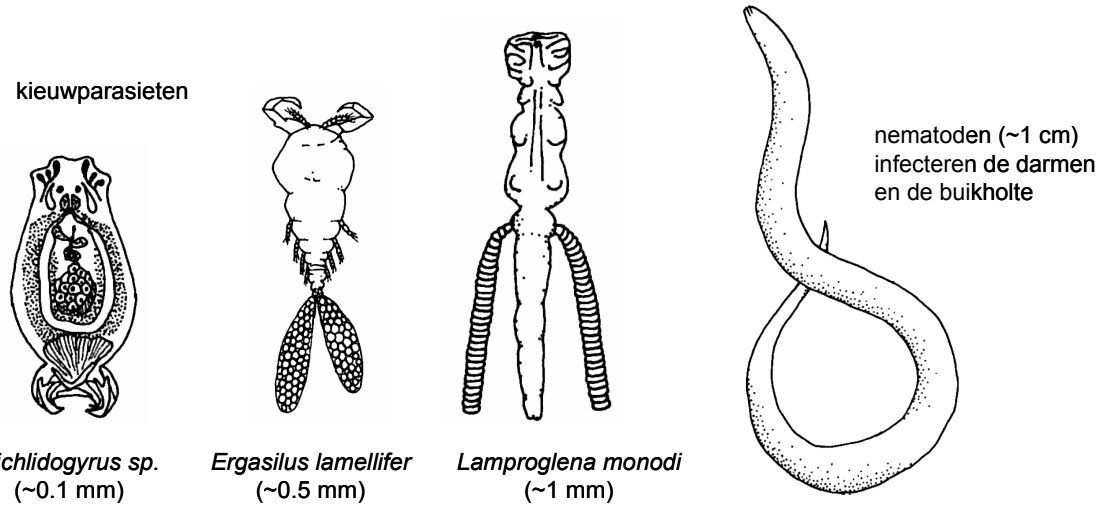
11 Reproductieve isolatie beschrijft de mate waarin groepen (soorten, populaties, varianten) met elkaar paren en levensvatbare, vruchtbare nakomelingen produceren. Wanneer paringen tussen groepen niet (meer) voorkomen, of geen vruchtbare nakomelingen opleveren, is reproductieve isolatie compleet.

In de Hoofdstukken 3, 4 en 5 heb ik onderzocht *waarom* vrouwtjes de kleur van een mannetje zo belangrijk vinden, en hoe het mechanisme dat kleurvoorkeur veroorzaakt zich verhoudt tot het splitsen van de *Pundamilia*'s in een blauwe en een rode variant. Eerst (**Hoofdstuk 3**) heb ik onderzocht of de rode kleur van de *P. nyererei* mannetjes een betrouwbare indicatie geeft van de kwaliteit van een mannetje.

Mijn waarnemingen, in dezelfde populatie als die uit Hoofdstuk 2, laten zien dat felrode mannetjes niet noodzakelijkerwijs dominant zijn over andere mannetjes: ze waren niet groter en hadden ook niet de grootste of best gelegen territoria. Vervolgens heb ik in een grote groep mannetjes het aantal parasieten geteld (Figuur 10.5), als maat voor de kwaliteit van het afweersysteem. Felrode mannetjes, en mannetjes met grote territoria, hadden minder parasieten dan andere mannetjes. Dit resultaat zou kunnen betekenen dat de voorkeur van vrouwtjes erop gericht is om vaders te selecteren die hun jongen 'goede genen' meegeven voor de afweer tegen parasieten. Het verband tussen kleur en immunologische afweer wordt misschien veroorzaakt door de beperkte beschikbaarheid van carotenoïden[12]. Carotenoïden spelen een rol bij de productie van gele, oranje en rode kleuren. Daarnaast vervullen ze belangrijke functies in het immuunsysteem. Een individu moet zijn beperkte voorraad carotenoïden daarom efficiënt verdelen over beide functies[13]. Het gevolg is dat alleen de *P. nyererei* mannetjes die hun immuunsysteem op orde hebben, zich kunnen veroorloven om carotenoïden te gebruiken om felle kleuren te maken. Dat verklaart het verband tussen kleur en parasieten.

Als vrouwtjes aan de rode kleur van een mannetje kunnen aflezen hoe goed zijn afweer tegen parasieten is, zou je verwachten dat alle vrouwtjes een voorkeur voor felrode mannetjes ontwikkelen. De voorkeur van *P. pundamilia*-vrouwtjes voor blauwe mannetjes is daarom moeilijk te begrijpen, tenzij ook de blauwe kleur van een mannetje informatie geeft over zijn kwaliteit. In **Hoofdstuk 4** heb ik daarom gekeken naar de parasieten van *P. pundamilia*. Ik heb gevonden dat ook in deze soort het aantal parasieten in een mannetje verband houdt met zijn kleur: felblauwe mannetjes waren het minst geïnfecteerd. Daarnaast bleek dat de twee soorten sterk verschillen in de besmettingsgraad van verschillende parasieten: *P. nyererei* had vooral veel parasitaire copepoden (roeipootkreeftjes) in de kieuwen, en *P. pundamilia* had veel nematoden (rondwormen) in de darmen. Deze verschillen hangen samen met de ecologische verschillen tussen de soorten: nematoden besmetten vissen via de uitwerpselen van vogels, die vooral in het ondiepe water vlakbij de rotsen terecht komen – de habitat van *P. pundamilia*. Copepoden daarentegen bereiken de kieuwen van hun gastheer rechtstreeks via het water. Hoe groter de waterstroom door de kieuwen, des te groter de besmettingskans.

12 Carotenoïden zijn in vet oplosbare verbindingen, die voorkomen in allerlei soorten groenten en fruit die oranje, geel, rood en groen van kleur zijn. Ze zijn genoemd naar wortels (carrots). Dieren kunnen zelf geen carotenoïden maken en moeten deze uit planten halen.
13 Een dergelijk dilemma noemen biologen een 'trade-off'.



Figuur 10.5 De vier meest voorkomende parasieten in *P. pundamilia* en *P. nyererei*.

Terwijl *P. pundamilia* vooral insecten eet, filtert *P. nyererei* zoöplankton uit het water en daarbij passeert voortdurend water de kieuwen.

Felle kleuren zijn dus in zowel *P. pundamilia* als *P. nyererei* betrouwbare indicators voor individuele kwaliteit, en de soorten verschillen in parasietenlading. Vrouwtjes van beide soorten doen er dus goed aan om de mooiste mannetjes van hun eigen soort te kiezen. Seksuele selectie voor goede genen (voor afweer tegen parasieten) versterkt zo de reproductieve isolatie tussen de soorten.

Toch lijkt het onwaarschijnlijk dat alléén seksuele selectie voor immunologische kwaliteit de splitsing van *P. pundamilia* en *P. nyererei* heeft veroorzaakt. Immers, individuen die intermediaire kenmerken hebben (wat betreft kleur, habitat, dieet enzovoort) zouden zich kunnen aanpassen aan besmettingsgraden van parasieten die tussen beide soorten in liggen. Dat betekent dat in plaats van twee gescheiden soorten, er één soort zou ontstaan die alle variatie van *P. pundamilia* en *P. nyererei* omvat, inclusief alle intermediaire fenotypen. In **Hoofdstuk 5** onderzoek ik daarom een ander mechanisme dat de reproductieve isolatie tussen *P. pundamilia* en *P. nyererei* wél zou kunnen veroorzaken: divergente seksuele selectie als bijproduct van natuurlijke selectie op de zintuigen.

Om op te vallen voor vrouwtjes, moeten haplochromine mannetjes zorgen dat hun kleuren contrasteren met de achtergrond. Het water van het Victoriameer is vrij troebel en groenig van kleur. Lange golflengtes (geel en rood licht) dringen verder door dan korte golflengtes (blauw licht). Daardoor zijn de lichtomstandigheden in diep water heel anders dan in ondiep water. Ik heb het licht in de habitats van *P. pundamilia* en *P. nyererei* gemeten en daaruit blijkt dat in de diepere habitat van *P. nyererei* nauwelijks blauw licht doordringt, maar wel geel en rood licht. Om op te vallen tegen de groenige achtergrond van het water, kunnen mannetjes in dieper water dus alleen maar gebruik maken van gele en rode kleuren – blauwe kleuren vallen hier niet op. Natuurlijke selectie zorgt ervoor dat vissenogen goed zijn aangepast aan hun omgeving. Als *Pundamilia* vrouwtjes ook zo zijn aangepast, zou dat betekenen dat de verschillende lichtomstandigheden op

verschillende dieptes hebben geleid tot verschillende aanpassingen van het visuele systeem. Deze verschillen kunnen de partnerkeuze beïnvloeden: gevoeligheid voor rood licht betekent dat rode voorwerpen als opvallend worden ervaren. In Hoofstuk 2 had ik al gevonden dat *P. nyererei* vrouwtjes een voorkeur hebben voor opvallende, felrode mannetjes. Misschien is deze voorkeur het gevolg van verhoogde gevoeligheid voor rood licht. Om deze hypothese te toetsen heb ik de kleurgevoeligheid van *Pundamilia* vrouwtjes onderzocht. Daarvoor heb ik een 'optomotor'-experiment toegepast, dat gebruik maakt van de neiging van vissen om bewegende lichtstimuli te volgen. Door zowel blauw als rood licht van verschillende sterkte te presenteren, kon ik vaststellen dat de soorten inderdaad verschillen in kleurgevoeligheid: *P. pundamilia* vrouwtjes waren gevoeliger voor blauw licht, en *P. nyererei* vrouwtjes waren gevoeliger voor rood licht. Deze resultaten suggereren dat de variatie in lichtomstandigheden op verschillende dieptes in het Victoriameer heeft geleid tot divergente selectie voor kleurgevoeligheid. Verschillen in kleurgevoeligheid oefenen op hun beurt divergente selectie uit op de kleuren van mannetjes. Dit mechanisme zou het ontstaan van *P. pundamilia* en *P. nyererei* kunnen verklaren, omdat de lichtomstandigheden in het meer ervoor zorgen dat intermediair gekleurde mannetjes niet opvallen, daardoor onaantrekkelijk zijn voor vrouwtjes en dus weinig nakomelingen produceren. Zo blijven alleen de extreem gekleurde mannetjes over.

Het Victoriameer wordt door eutrofiëring[14] steeds troebeler. Dat heeft gevolgen voor de diversiteit van haplochromine cichliden, omdat hybridisatie tussen soorten toeneemt in troebel water. Dat geldt ook voor *P. pundamilia* en *P. nyererei*. In **Hoofdstuk 6** heb ik onderzocht hoe het verband tussen transparantie en hybridisatie ontstaat. Waarschijnlijk kan partnerkeuze op grond van visuele kenmerken in troebel water niet goed plaatsvinden. Maar dat kan op twee verschillende manieren werken: i) partnervoorkeuren variëren met de troebelheid van het water; of ii) partnervoorkeuren zijn hetzelfde in troebel of helder water, maar in troebel water kunnen vrouwtjes de verschillen tussen mannetjes gewoon niet goed zien. Daardoor paren ze, ondanks hun voorkeur voor felgekleurde mannetjes van hun eigen soort, toch met mannetjes met de verkeerde kleur. Om deze alternatieven te onderscheiden, heb ik het partnerkeuze-gedrag van twee verschillende populaties van *P. nyererei* met elkaar vergeleken. Ik heb een tweede serie partnerkeuze-experimenten uitgevoerd, zoals eerder beschreven in Hoofdstuk 2, maar nu met een *P. nyererei* populatie uit troebel water. Vrouwtjes uit deze populatie bleken minder belang te hechten aan de rode kleur van de mannetjes; daarentegen hadden ze een voorkeur voor felgele en voor grote mannetjes. De kenmerken van de mannetjes uit troebel water passen daarbij: deze zijn minder rood en meer oranje, en groter, dan in helder water. Deze resultaten geven aan dat het

14 Eutrofiëring: het toenemen van de hoeveelheid voedingsstoffen in een bepaald milieu. Vaak wordt eutrofiëring door menselijke activiteiten veroorzaakt (bemesting van grasland, lozen van afvalwater) en in natuurlijke systemen is dat niet altijd wenselijk. In het Victoriameer bijvoorbeeld leidt eutrofiëring tot algenbloei en neemt de helderheid van het water af.

verband tussen de helderheid van het water en de mate van hybridisatie niet louter het gevolg is van omgevingsvariatie, maar dat het partnerkeuzegedrag van vrouwtjes zich aan de plaatselijke omstandigheden heeft aangepast. Naast dit effect op partnerkeuze, heeft afnemende transparantie ook gevolgen voor de ecologische verschillen tussen soorten: *P. nyererei* leeft in dieper water als het water helder is. Die verschuiving is een aanwijzing dat de 'ecologische ruimte' in troebel water kleiner wordt, met mogelijke gevolgen voor het aantal soorten dat naast elkaar kan voorkomen. Deze resultaten onderstrepen het belang van maatregelen tegen de verdere eutrofiëring van het Victoriameer.

Hoofdstukken 7 en 8: de kleuren van *Neochromis omnicaeruleus*

Soortsvorming door divergente seksuele selectie begint met variatie in kleur en kleurvoorkeur *binnen* soorten. Als model voor dergelijke intraspecifieke[15] kleurvariatie heb ik *Neochromis omnicaeruleus* onderzocht, een van de meest polymorfe haplochrominen. Naast het blotch polymorfisme, onderwerp van Hoofdstuk 8, vertoont het niet-geblokte fenotype aanzienlijke kleurvariatie: van bruingrijs tot zonnegeel in vrouwtjes, en van metallic blauw, via groen, tot geel met rood in mannetjes (Figuur 7.1, pagina 113).

Deze intraspecifieke variatie lijkt op de kleurverschillen die tussen verschillende soorten haplochrominen bestaan, en zou daarom misschien het startpunt voor divergente selectie kunnen zijn, die uiteindelijk tot soortsvorming leidt. In **Hoofdstuk 7** heb ik daarom de kleurvariatie van *N. omnicaeruleus* onderzocht. Ik heb gevonden dat gele mannetjes gemiddeld kleiner waren dan blauwe of groene mannetjes, en in meerderheid niet geslachtsrijp. In vrouwtjes bestonden dergelijke verschillen tussen kleurtypen niet. Vervolgens heb ik kruisingsexperimenten gedaan in het aquarium, die lieten zien dat kleur genetisch bepaald is: gele ouders kregen meer gele jongen dan blauwe ouders. Tenslotte bleek uit aquariumobservaties dat gele mannetjes blauw werden wanneer ze geslachtsrijp waren, en dat deze verandering vaak samenging met het bereiken van een dominante positie. Het feit dat kleurexpressie in *N. omnicaeruleus* een genetische achtergrond heeft, betekent dat de blauw/geel variatie zou kunnen reageren op selectie. Aan de andere kant maakt de schaarste aan geslachtsrijpe gele mannetjes in het veld het onwaarschijnlijk dat vrouwtjes een voorkeur voor deze mannetjes ontwikkelen. Daarom concludeer ik dat in de huidige situatie, divergente seksuele selectie op kleurvariatie niet snel zal leiden tot reproductieve isolatie tussen blauwe en gele *N. omnicaeruleus*.

Naast de blauwe en gele fenotypen, komen in *N. omnicaeruleus* twee 'blotch' typen voor: 'orange-blotched' en 'white-blotched' (Figuur 8.4, pagina 128). Vergelijkbare blotch polymorfismen bestaan in verschillende andere haplochromine cichliden. In de meeste gevallen zijn blotched vrouwtjes veel talrijker dan blotched mannetjes. In *N. omnicaeruleus* kan die scheve verdeling deels verklaard worden door een genetische correlatie tussen het blotch fenotype en geslachtsbepalende

15 Binnen soorten: *intra* specifiek; tussen soorten: *inter* specifiek.

genen: blotched individuen dragen een dominante genetische factor die blotched mannetjes in vrouwtjes verandert. Kruisingen in het laboratorium gaven echter aan dat, ondanks dit genetische effect, blotched mannetjes veel talrijker zouden moeten zijn dan we in de natuur zien. Omdat het blotched kleurpatroon heel opvallend is, en omdat mannetjes zich vaak opvallender gedragen dan vrouwtjes, heb ik in **Hoofdstuk 8** de hypothese onderzocht dat blotched mannetjes uit het meer verdwijnen door een verhoogd predatierisico. Ik heb een experiment uitgevoerd met ijsvogels, waaruit bleek dat blotched vissen inderdaad als eerste werden gevangen. Verder heb ik in onderwater-observaties vastgesteld dat mannetjes opvallender gedrag vertoonden dan vrouwtjes. Deze resultaten ondersteunen het idee dat blotched mannetjes, in vergelijking met andere mannetjes en met vrouwtjes, een hoger risico op predatie lopen.

Als vogels inderdaad vooral veel blotched mannetjes uit de populatie halen, moet je het effect daarvan in de populatiestructuur kunnen zien: redelijke aantallen blotched mannetjes bij de kleintjes, maar geleidelijk afnemende aantallen naarmate de vissen groter worden. Deze voorspelling kon ik niet bevestigen: blotched mannetjes waren in alle grootteklassen zeer schaars. Mijn resultaten zijn dus enigszins tegenstrijdig. Het lijkt onwaarschijnlijk dat verschillen in predatie ervoor zorgen dat blotched mannetjes al op jonge leeftijd uit de populatie verdwijnen, want gedragsverschillen tussen mannetjes en vrouwtjes ontstaan pas als de vissen geslachtsrijp zijn. Misschien worden blotched mannetjes slechts in lage aantallen geproduceerd, en is de genetische achtergrond van het blotch polymorfisme ingewikkelder dan we dachten. Mijn onderzoek lost het mysterie van de blotched mannetjes dus niet op. Wel geef ik het eerste bewijs dat opvallende kleurpatronen in cichliden het risico op predatie kunnen verhogen. Daarmee suggereren mijn resultaten dat natuurlijke selectie belangrijke gevolgen zou kunnen hebben voor het ontstaan en voortbestaan van kleurvariatie in deze vissen.

Hoofdstuk 9: Synthese

Het laatste hoofdstuk geeft een samenvatting van de belangrijkste resultaten van mijn werk en hun betekenis voor het onderzoek naar haplochromine soortsvorming. Ik concludeer dat de evolutie van haplochromine kleurpatronen door verschillende factoren wordt beïnvloed. Kleuren zijn kostbaar, omdat ze het risico op predatie vergroten en omdat ze energie vergen die anders geïnvesteerd zou worden in belangrijke fysiologische processen, zoals afweer tegen parasieten. Omdat alleen individuen van hoge kwaliteit zich dergelijke kosten kunnen veroorloven, zijn felle kleuren eerlijke signalen van individuele kwaliteit. Haplochromine kleuren kunnen daardoor onderhevig zijn aan seksuele selectie voor ‘goede genen’. Variatie in omgevingsfactoren, zoals lichtomstandigheden en blootstelling aan parasieten, zorgt ervoor dat niet alle vrouwtjes dezelfde voorkeur hebben. Dat betekent dat seksuele selectie voor genetische kwaliteit aan soortsvorming kan bijdragen. Daar staan twee mechanismen tegenover die kleurendiversiteit juist beperken. Hoge predatiedruk kan het ontstaan en voortbestaan van opvallende kleurpatronen in de weg staan, zodat nieuwe kleurtypen niet talrijk genoeg worden om

tot soorten uit te groeien. Bovendien wordt diversiteit bedreigd door de eutrofiëring van het Victoriameer. Afnemende helderheid van het water kan het partnerkeuzegedrag van vrouwtjes zodanig beïnvloeden dat ook minder felgekleurde mannetjes zich voortplanten. Dat heeft nadelige gevolgen voor het behoud van kleurverschillen tussen soorten en kan uiteindelijk leiden tot een toename van het aantal paringen tussen verschillende soorten, waardoor ze kunnen samensmelten en de diversiteit afneemt. Tenslotte wordt de ecologische ruimte in troebel water kleiner, zodat er minder soorten naast elkaar kunnen blijven bestaan.

Natuurbehoud vergt inzicht in de biologische processen die biodiversiteit bepalen. Hoewel mijn proefschrift vooral fundamentele vragen beantwoordt over haplochromine soortsvorming in het Victoriameer, hoop ik dat mijn resultaten uiteindelijk zullen bijdragen aan het behoud van dit bijzondere ecosysteem.

Lotgenoten, vrienden, promotores en reviewers ten spijt, uiteindelijk is het schrijven van een proefschrift een eenzame bezigheid. Dat neemt niet weg dat sommige mensen behulpzaam of zelfs onmisbaar zijn geweest bij de totstandkoming van dit boekje en zij zullen hier vermeld worden.

Mijn doctoraalonderwerpen bij Ton Groothuis en Michael Taborsky vormden een ideale voorbereiding op mijn promotieonderzoek. Ton heeft me laten zien hoe leuk het is om aan cichliden te werken en toonde ook tijdens dit project een kritische betrokkenheid die het boekje ten goede kwam. Michael taught me how to study cichlids in the field and these lessons in Lake Tanganyika have greatly facilitated my work in Lake Victoria.

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My 'counterpart' Hillary John Mrosso has been a very important figure during my fieldwork in Tanzania. He introduced me to the peculiarities of Tanzanian culture, shared responsibilities and joined me in adventures that I would never have survived on my own. I remember an anxious midnight in a Nairobi bus station, where Mrosso protected our research equipment with his life. There were also many hours, days and weeks that we walked the dusty streets of Mwanza, Arusha and Dar es Salaam together, in our quest for permits, visa, tax exemptions and numerous other logistical formalities. I thank him for his help and companionship and I wish him good luck!

Throughout my fieldwork, the Tanzanian Fisheries Research Institute (TAFIRI) has been very helpful and welcoming. I would like to thank especially its Director Prof. Philip Bwathondi, and Mr. Egid Katunzi, Director of the Mwanza Centre in Nyegezi, for their assistance. The Tanzanian Commission for Science and Technology (COSTECH) provided me and my students with the necessary permits to conduct our research.

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Several MSc students have contributed to the fieldwork. Linda Söderberg (Chapter 2) used her extensive diving experience for many hours of under water observations - unfortunately her diving suit was not up to the challenge. Brechtje Eshuis (Hoofdstuk 8) hád helemaal geen duikpak, maar met de haar kenmerkende creatieve toewijding was dat snel in elkaar geflanst. Dankzij haar engelengeduld overwonnen de ijsvogels hun schroom, en aten ze onze vijvers leeg. Erwin Ripmeester (Hoofdstuk 2) was weliswaar niet in Nyegezi, maar heeft zich over de vaderschapsanalyse van een veldexperiment gebogen. Met grote vastberadenheid worstelde hij maandenlang met kansloze AFLP's, om uiteindelijk met de microsatellieten van Martin Taylor in Norwich de familiebanden te ontrafelen.

Marcel Haesler joined the team as a field assistant for the work on *N. omnicaeruleus* (Chapters 7 and 8). His unwavering positivism made us realise how lucky we were.

Other students took part in the laboratory work in Leiden. Lisa Johnson (Chapter 2) did the first mate-choice experiments for this thesis and proved to be an excellent fish observer. A large part of the work of Chapter 3 is the result of the work of Jeanette Biemans, Paloma Jimenez and Michael van der Spoel. They dissected lots of *Pundamilia*'s for parasite load analysis and displayed an admirable dedication and patience. Tot slot zou Hoofdstuk 4 niet zijn geschreven zonder Anne van Rooijen.

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Martine

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1998	Veldassistent bij Michael Taborsky in Mpulungu.
1998-2000	Onderzoeks- en beleidsmedewerker bij de Koninklijke Luchtmacht, Sectie Natuurtechniek en Ecologisch Beheer. Radaronderzoek naar vogelmigratie; evaluatie van vogel-verjaagmethoden op vliegbases.
2000-2006	Promotieonderzoek bij de Sectie Dierecologie van het Instituut voor Biologie Leiden (IBL). Promotores: Jacques van Alphen en Ole Seehausen. Veldwerk in Tanzania (14 maanden); labwerk en data-analyse in Leiden. De resultaten van dit onderzoek worden beschreven in dit proefschrift. Daarnaast heb ik mijn werk gepresenteerd tijdens seminaria en voordrachten op internationale congressen in Zurich, Pennsylvania State College, Montreal, Leeds, Leiden, Bern, Jyväskylä, Krakow, Fribourg en Amsterdam. In 2003 heb ik in Leiden een congres georganiseerd voor jonge evolutionair biologen, over 'Sex, Sexual Selection and Speciation'. Daarna heb ik een symposium georganiseerd tijdens het ESEB congres in Leeds (2003), en maakte ik deel uit van de Commissie Wetenschappelijke Bijeenkomsten van het IBL (2003-2004).
2006-	Deze zomer begin ik aan een door NWO (Rubicon) gefinancierd postdocproject bij Molly Cummings aan de Universiteit van Texas (Austin).

In peer-reviewed journals

Maan M.E., Hofker C.D., Van Alphen J.J.M. and Seehausen O. Sensory drive in cichlid speciation. *American Naturalist* 167, in press.

Dijkstra P.D., Gricar B.L.A., Seehausen O., Maan M.E. and Groothuis T.G.G. 2006. Can male-male competition stabilise speciation? A test in Lake Victoria haplochromine cichlid fish. *Behavioral Ecology and Sociobiology* 59, 704-713.

Maan M.E., Haesler M.P., Seehausen O. and Van Alphen J.J.M. 2006. Heritability and heterochrony of polychromatism in a Lake Victoria cichlid fish: stepping stones for speciation? *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* 306B, 168–176.

Maan, M.E., Seehausen, O., Söderberg, L., Johnson, L., Ripmeester, E.A.P., Mrosso, H.D.J., Taylor, M.I., Van Dooren, T.J.M. and Van Alphen, J.J.M. 2004. Intraspecific sexual selection on a speciation trait, male coloration, in the Lake Victoria cichlid *Pundamilia nyererei*. *Proceedings of the Royal Society of London, Series B: Biological Sciences* 271, 2445-2452.

Maan, M.E., Groothuis, T.G.G. and Wittenberg, J. 2001. Escalated fighting despite predictors of conflict outcome: solving the paradox in a South-American cichlid fish. *Animal Behaviour* 62, 623-634.

Manuscripts submitted

Maan M.E., Eshuis B., Haesler M.P., Schneider M.V., Seehausen O. and Van Alphen J.J.M. Sex reversal, differential predation and incomplete speciation in a polymorphic Lake Victoria cichlid.

Maan M.E., Van der Spoel M., Quesada Jimenez P., Van Alphen J.J.M. and Seehausen O. Fitness correlates of male coloration in a Lake Victoria cichlid: evidence for parasite-mediated sexual selection on a speciation trait.

Maan M.E., Van Rooijen A.M.C., Van Alphen J.J.M. and Seehausen O. Parasite-mediated sexual selection and species divergence in Lake Victoria cichlid fish.

Maan M.E., Van Alphen J.J.M. and Seehausen O. Female mating preferences and male coloration co-evolve in response to water transparency in a Lake Victoria cichlid fish.

Other publications

Maan, M.E. and Verzijden, M.N. 2005. Seksuele selectie en soortsvorming. *Cichlidae, bi-monthly periodical of the Netherlands Cichlid Association* 31, 132-141.

Van Belle, J., Maan, M.E., Van Gasteren, H. and Buurma, L.S. 2000. Using radar to obtain a quantitative description of summer bird movements in the Dutch coastal area. *Proceedings of the 25th Meeting of the International Bird Strike Committee*, WP-RS2, 443-460.

'This is an elegant study with implications for our understanding of divergent sexual selection on male colour patterns in haplochromine cichlids.'

'I am not convinced.'

'The statistical analyses seem generally robust, and the field work rigorous.'

'The experimental set-up is poor, and the interpretation and presentation of the data is weak.'

'This system is very complicated.'

'…. well written with careful attention to detail.'

'I must admit I found it a rather difficult read.'

'My suggestion would be to add more data or to do some experimental work to increase the reliability of the findings.'

'By no means is the collection of additional data called for.'

'The study design and statistical analyses are sound, and the authors are commended on the difficulty of obtaining these sorts of important field data.'

'I have serious doubts about the validity of the interpretation of the data and the conclusions drawn.'

'Extremely interesting….. very timely and sure to attract attention.'

'I found it a bit confusing.'

'You can seek publication elsewhere.'

'Some interesting bits and pieces in this work….'

'The contradiction between experimental prediction and real world observation is enough for me.'

'This is quite unconventional.'

'Yeah!'