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Speciation and Radiation in African Haplochromine Cichlids

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8.1 Introduction

The explosive radiation of cichlid fishes in the African Great Lakes has intrigued biologists for many decades. The faunas of these lakes are outstanding, both in species richness and in the composition of their fish fauna. Several of these lakes contain as many or even more fish species than all the rivers and lakes of Europe together (Lowe-McConnell 1987; Kottelat 1997). About 90% of the fish species in each lake belong to a single family, the cichlids (Cichlidae; Teleostei) and are endemic to that lake. Estimates of the phylogenies of these species flocks suggest that the species of Lakes Victoria, Malawi, and Tanganyika have evolved *in situ* (Meyer *et al.* 1990; Lippitsch 1993; Nishida 1997). Even more remarkable, for Lakes Malawi and Victoria the species flocks are derived from one or only a few closely related ancestral species and are all haplochromines. In comparison to the diversity of these lakes, riverine cichlid fish faunas in Africa and South America are considerably less diverse.

The unusually fast ecological radiation of haplochromine cichlids and the exceptionally dense species packing of these fishes demands an understanding of what is so special about them. Most lacustrine species flocks of other fish taxa, even other cichlid taxa, are less diverse in ecology and species numbers. The versatility of the pharyngeal jaw apparatus, physiological properties, and their mouthbrooding behavior may all be necessary attributes, but these alone are not sufficient to explain the exceptional diversification of haplochromines. In this chapter, we argue that it is the combination of a number of factors.

Of the three African great lakes, Lake Tanganyika is the oldest and its age has been estimated to be between 9 and 12 My (Cohen *et al.* 1993). Lake Malawi's age has been estimated to be 1–2 My (Fryer and Iles 1972). Lake Victoria is the youngest (0.25–0.75 My: Fryer 1997). Most likely it dried up 200 000 years ago (Martens 1977) and seems to have dried up again in the late Pleistocene, after which it refilled between 15 500 (Beuning *et al.* 1997) and 14 600 years ago (Johnson *et al.* 1996a). Paradoxically, the young Lakes Victoria and Malawi contain more endemic species (between 500 and 1000 each) than the old Lake Tanganyika, with about 250 species. In particular, the Lake Victoria species flock must have been the result of truly explosive speciation, even if the lake did not completely dry out during the late Pleistocene.

One theory about the rapid speciation in Lake Victoria is the isolation of cichlids in marginal lagoons (satellite lakes), when the water level was low. These smaller water bodies were created and reunited repeatedly, owing to fluctuations in the lake level. Allopatric speciation during isolation would contribute new species to the flock of the main lake when the water table rose again. New geological evidence (Johnson *et al.* 1996b, 2000; Beuning *et al.* 1997) suggests that Lake Victoria is merely 3.5 times older than Lake Nabugabo, the prime example of a satellite lake, which has persisted in a stable state for about 4000 years. Yet Lake Nabugabo contains only five endemic species. Hence, it cannot have contributed much to the diversity of over 500 species that originated in a period of possibly only 15 000 years.

Some species may have recolonized Victoria, after the Pleistocene drought, from Lake Edward, Lake Kivu, or the Malagarasi River. However, these must still have undergone explosive radiation within the Victoria lakebed to account for the large number of species today, because Lake Victoria does not share any species with these ecosystems and only a few of its many genera. Similarly, evolution of the species flock in Lake Malawi was attributed to allopatric speciation. Because Lake Malawi has probably not been split into separate lakes and there are few satellite lakes along its shore, it was suggested that the origin of the flock was by microallopatric speciation in habitat patches within the lake (Fryer and Iles 1972; Ribbink *et al.* 1983). This hypothesis assumes that water-level fluctuations caused periodic losses of suitable habitat and forced populations to colonize newly formed habitats (Arnegard *et al.* 1999).

However, the evidence that habitat stenotopy limits dispersal, a prerequisite for microallopatric speciation, is equivocal. Molecular data on population genetics suggest very low dispersal rates between occupied habitat patches (van Oppen *et al.* 1997; Arnegard *et al.* 1999), but artificial reefs are rapidly colonized, with the first immigrants representing the most species-rich genera (McKaye and Gray 1984). Moreover, in both Lakes Victoria and Malawi, even those species that live in continuous habitats have speciated rapidly (Turner 1996; Shaw *et al.* 2000). Microallopatric population differentiation is known to occur within Lake Victoria (Seehausen 1996; Witte *et al.* 1997; Seehausen *et al.* 1998; Bouton *et al.* 1999), which probably has resulted in speciation among littoral cichlids (Seehausen and van Alphen 1999). It is, however, difficult to explain the explosive rate of speciation and ecological diversification as a product of microallopatric or parapatric speciation alone. The sympatric occurrence of sibling species and color morphs, often on a small number of neighboring islands or even a single island, suggests that at least part of the speciation in Lake Victoria might occur in sympatry. One reason why investigators are skeptical about sympatric speciation is that it is hard to prove (Schilthuisen 2001). This is because it can be demonstrated only on the basis of a painstaking analysis of all the steps in the speciation process and because data on distribution alone do not provide a convincing proof (Seehausen and van Alphen 1999, 2000). In this chapter all the present-day evidence that supports

the notion that some of the speciation in the haplochromines occurs in sympatry is presented.

In particular, the evidence for the hypothesis that sexual selection by mate choice for male coloration is the driving force behind the generation and reproductive isolation of color morphs of Lake Victoria haplochromines is reviewed. Note that no postzygotic isolation, in the form of reduced hybrid fertility, or viability between species of these fishes exist. The polygynous breeding system of haplochromines is associated with strong asymmetric investment in parental care, in which the females invest much more than males. Such a breeding system is conducive to sexual selection on male characters. We suggest that sexual selection, together with disruptive selection on feeding and on other characters, may have led to the present species diversity.

A second hypothesis discussed in this chapter is that a gene that causes sex reversal can cause speciation in haplochromines. The presence of this gene has probably resulted in the evolution of some of the mate preferences observed, and could be at the basis of the cascade of events that led to speciation by sexual selection upon coloration.

We show that genetic isolation caused by disruptive sexual selection is accompanied or followed by ecological diversification that results in niche differentiation between species. The striking diversity of feeding niches that characterizes cichlids of Lake Victoria suggests that niche differentiation occurred by rapid specialization for different feeding niches. Finally, we try to indicate why cichlids have shown adaptive radiation more often than other percoid relatives and what allows cichlids to specialize rapidly for different feeding niches.

8.2 Sexual Selection and Speciation in Cichlids

The reproductive behavior of haplochromines, the frequent sexual dimorphism in coloration, the polygynous mating system, and the male display during courtship in haplochromines suggest that sexual selection by female mate choice for male coloration could play an important role in the evolution of these fish.

The breeding system

The large species flocks of Lakes Malawi and Victoria consist of haplochromine cichlids. Haplochromines have colorful and conspicuous males, while the females of many species are cryptically colored. Haplochromines have a polygynous breeding system with female parental care by mouth brooding and, in many species, guarding of young fry. Females with ripe eggs visit males on their territories, where males initiate courtship. A courtship bout begins with the male approach, followed by a lateral display by the male, during which it displays its body and fin coloration by posing in front of the female with an erected dorsal fin. If the female remains or approaches the male, he quivers with the body, with the dorsal fin partly folded and the anal fin stretched out. This is followed by lead swimming to the spawning pit. There the male presents its egg dummies on the anal fin while circling in the pit.

Sexual selection and breeding system

Some researchers have suggested that mating preferences could be instrumental in speciation of cichlids. Kosswig (1947) suggested that mating preferences could lead to the isolation of family groups in cichlids, Fryer and Iles (1972) and Dominey (1984) suggested that microgeographic divergence in courtship might have been important in speciation of monogamous taxa. However, speciation rates in monogamous lacustrine cichlids are not higher than in other lacustrine fish that lack pair bonds and complex courtship. If anything, the evidence suggests the contrary: the most species-rich lineages of cichlids are polygynous. Until recently, studies on the functions of sexual dimorphisms in polygynous cichlids have been few, and few investigators have related these dimorphisms to speciation. McElroy and Kornfield (1990) studied male courtship behavior in Malawi cichlids and found that it was highly conserved among closely related species, and hence unlikely to have played a role in speciation.

Traits on which sexual selection could operate

Males of some cichlids on sand bottoms in Lakes Tanganyika and Malawi form lekks and construct crater-shaped spawning sites from sand. McKaye (1991) hypothesized that sexual selection on these extended phenotypes could lead to speciation, but there is no direct evidence for this hypothesis. Hert (1989) demonstrated that females of a rock-dwelling Malawi cichlid prefer males with egg dummies on their anal fin to males from which the egg dummies are removed. Goldschmidt (1991) demonstrated that interspecific variation in egg dummy size correlated negatively with light intensity in the habitat. Goldschmidt and Visser (1990) suggested that sexual selection on egg-dummy morphology could lead to speciation. Again, no direct evidence in support of this hypothesis is available. Male display during courtship suggests that dorsal fin and body coloration also play important roles in mate choice. At least two studies have analyzed hue variation in male cichlids (McElroy *et al.* 1991; Deutsch 1997). In cichlid evolution, the appearance of male nuptial coloration is generally preceded by that of a polygynous mating system (Seehausen 2000). Some studies have found circumstantial evidence to support speciation by selection on male nuptial coloration (Marsh *et al.* 1981; McKaye *et al.* 1982, 1984). However, experimental tests of this hypothesis were not undertaken until recently (Seehausen *et al.* 1997; Knight *et al.* 1998; Seehausen and van Alphen 1998).

Such a test involves a number of steps:

- Provide evidence that female mate choice is based on male coloration.
- Provide evidence that across species female preference for male coloration is correlated with male coloration.
- Show that female preference can act as a genetic barrier between populations that differ in preference and color.
- Provide evidence that within-species variation exists for male coloration and female preference.

8.3 Sexual Selection in *Pundamilia*

Seehausen and van Alphen (1998) tested some of the assumptions discussed in Section 8.2 using two sibling species of the genus *Pundamilia*. *P. nyererei* and *P. pundamilia* are two anatomically very similar forms that behave as biological species in places where they occur sympatrically, except in places with exceptionally low water transparency where they interbreed (Seehausen *et al.* 1997). They usually show some ecological differences (Seehausen 1997; Seehausen and Bouton 1997). They can be distinguished by their male nuptial coloration. Males of *P. nyererei* are bright crimson dorsally, yellow on the anterior flanks, and their dorsal fin is crimson. Males of *P. pundamilia* are grayish white dorsally and on the flanks, and have a metallic blue dorsal fin (see Plate 1, lower part). Subtle differences in head anatomy help to distinguish the otherwise very similar females.

Evidence for sexual selection on male coloration

The behavioral responses of females to males of both species were studied in choice experiments under either white light or monochromatic light:

- Females of both species exhibited strong species assortative mate choice under white light. However, in tests under monochromatic light, when the interspecific differences in male coloration were masked, no preference was observed (Figure 8.1).
- Hybrids between *P. nyererei* and *P. pundamilia* can be obtained easily under no-choice conditions in the aquarium. Intermediate phenotypes resembling F1 or F2 hybrids were not found in most of their common geographic range, but occurred in abundance at several places with exceptionally low water transparency.

In summary, experimental evidence and field observations show that female mate choice based on male coloration prevents interbreeding when the light conditions allow discrimination, but that hybridization occurs when male coloration cannot be assessed by the female. This experiment thus provides evidence that female choice of male coloration can be responsible for reproductive isolation between existing species.

Field evidence from comparisons between communities

Field evidence supports the experimental evidence: Seehausen *et al.* (1997) reasoned that if sexual selection on coloration plays a role in reproductive isolation between species, ambient light conditions could set limits to the action of sexual selection. Sexual selection can only operate on variation in male coloration and in mate preference where light conditions allow the perception of the color variation. They predicted that the extent of interspecific color variation, the number of species, and the number of color morphs within a species should increase with the width of the ambient light spectrum.

Data collected at two series of islands to test these hypotheses showed that the ambient light spectrum explained the variation in these parameters better than any

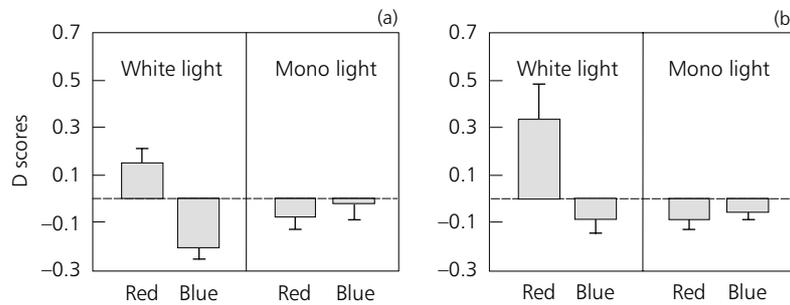


Figure 8.1 Mean difference scores and standard error of female mate choice [responsiveness (= proportion of encounters in which the male display was followed by a female approach or follow) to red male minus responsiveness to blue male] under white and monochromatic light. (a) Approaching a male; (b) following a male. Each bar is based on the mean scores from four females, each tested three times (red mate choice of “red” females, blue mate choice of “blue” females).

of a large number of other ecological variables. The distinctiveness of nuptial-hue difference between sympatric species, the number of sympatric color morphs within a species, and the number of coexisting species within genera increased with the width of the ambient light spectrum. Moreover, the brightness and distinctiveness of male coloration had decreased in the populations within the previous ten years, following a decrease in water transparency through eutrophication. Finally, the frequency of phenotypes that are intermediate between sympatric forms was correlated negatively with water transparency. These observations are all consistent with sexual selection by direct mate choice as the principle factor that keeps closely related species genetically isolated.

Intrapopulation variation in preference and color

To see whether intrapopulation variation in preferences for male nuptial coloration exists in *Pundamilia*, females of one population were tested. In this population the males vary between blue and dull red in the dorsal fin and between blue gray and dull red on the dorsum. Whereas most females consistently and significantly preferred blue over red, some were unselective and others tended to prefer red males. This demonstration that intraspecific variation for male coloration co-occurs with intraspecific variation in female preference (Seehausen 2000) shows that intraspecific disruptive sexual selection on coloration can play a role in the evolution of color polymorphisms. Such color polymorphisms could result in speciation.

8.4 Sexual Selection in *Neochromis omnicaruleus*

To investigate if and how sexual selection acts on color variation within a species, Seehausen *et al.* (1999) studied the polymorphic species *N. omnicaruleus*. If morphs would mate selectively, color polymorphisms might represent incipient

stages in speciation by disruptive sexual selection. Field observations and laboratory experiments suggest that *N. omnicaeruleus* represents an original species with blue males and brown females, and two incipient species that are black and orange blotched or black and white blotched. Seehausen *et al.* (1999) chose this species because evidence suggested that counteracting selection has retarded the speciation of the blotched morphs, which would allow the study of what otherwise might be short-lived transient stages of speciation. They tested whether a polymorphism has the properties of an incipient stage in speciation, and whether mating preferences can disrupt gene flow in sympatry prior to ecological differentiation, by investigating:

- Ecological correlates of sympatric color variation;
- Genetics of polymorphic color variation;
- Frequency of color phenotypes in the natural population;
- Female and male mate choice in aquarium experiments.

Morphometric differences

Wild-type *N. omnicaeruleus* differs from its close relative *N. rufocaudalis* by a smaller cheek depth, larger eyes, and a longer mandible. The larger cheek depth and smaller eyes leave more space for the adductor mandibulae muscles. Together with the shorter jaws, this provides *N. rufocaudalis* with more biting power than *N. omnicaeruleus* and makes *N. rufocaudalis* a more specialized algae scraper. While these differences occur between closely related and ecologically very similar species, no morphometric differences were found between color morphs of *N. omnicaeruleus*.

Genetics of color variation

According to Kornfield (1991), haplochromines have XY sex determination with the male as the heterogametic sex. Fish do not have dimorphic sex chromosomes. Nevertheless, in the following the chromosome that carries the main sex-determining genes is called the sex chromosome and the others autosomes.

In nature, nine different phenotypes of females were found and four male phenotypes (see Plate 1, Figure 8.2, and Table 8.1). The most common, “plain”-type (P), has females that are yellow–brown with dark vertical bars and males that are bright metallic blue. The other common types are either “white and black blotched” (WB), or “orange blotched” (OB). The other types are intermediates between these three, as well as almost entirely black and almost entirely orange, and occur in low frequencies.

Color in *N. omnicaeruleus* is associated with sex modifiers. WB and OB are associated with a dominant X-linked sex reversal gene. The presence of a recessive autosomal male determiner (the “male rescue gene”) suppresses the dominance of this gene over the y-allele. This rescue gene is associated or identical with the modifiers of WB and OB coloration. Tight linkage between sex determiners and color genes has been found in other fish as well. OB and/or WB color morphs occur in haplochromines in several African lakes and are usually associated with a

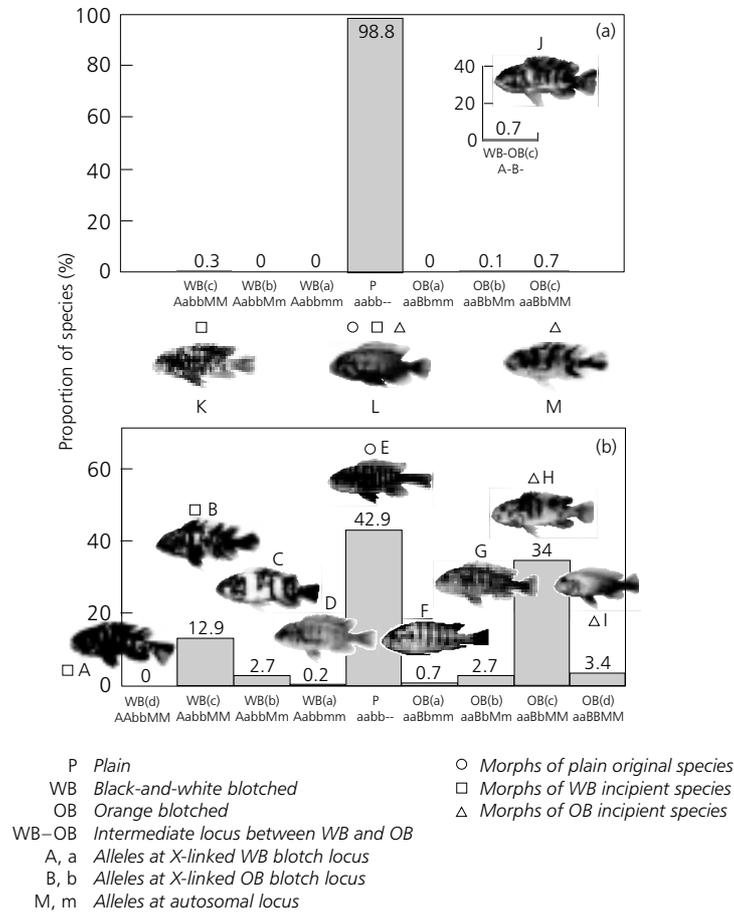


Figure 8.2 The color polymorphism in *N. omnicaeruleus* (see Plate 1 for the morphs in color, identified by letter). Phenotypes, genotypes, and natural frequencies of the phenotypes: (a) males; (b) females. M in the genotype notations represents two recessive male rescue alleles and additive blotch expressivity modifiers M_A and M_B (see text). The three common P phenotypes in both sexes are those from P parents, WB(c) mothers, and OB(c) mothers. They differ at the autosomal locus and in mating preferences. Female and male morphs with mutually compatible mating preferences are designated by symbols. Information on mating preferences within and between the original species and the WB incipient species is based on the reported mate-choice experiments. That preferences within and between the original species and the OB incipient species parallel those in the WB system is an assumption supported by experiments with a wild OB(c) female that preferred blue over WB males, but did not discriminate between a blue and an OB male (unpublished), and by field underwater observations. Courtship between OB(c) females and P males has been observed on many instances, and a focal observation (20 minutes) of one OB male revealed courtship only with an OB female, although three P, three WB, and two OB females were encountered by that male.

Table 8.1 Possible genotypes and the resultant phenotypes found in nature. The suffixes (a) to (d) indicate increasing blotchiness.

Genotype	aabb	Aabb	AAbb	aaBb	aaBB	AaBb	AaBB	AABb	AABB
<i>Females</i>									
mm	P	WB(a)	–	OB(a)	–	–	–	–	–
mM	P	WB(b)	–	OB(b)	–	–	–	–	–
MM	P	WB(c)	WB(d)	OB(c)	OB(d)	WB/OB(c)	–	–	–
<i>Males</i>									
mm	P	–	–	–	–	–	–	–	–
mM	P	–	–	OB(b)	–	–	–	–	–
MM	P	WB(c)	–	OB(c)	–	–	–	–	–

strong female determiner (Fryer and Iles 1972; Holzberg 1978; Lande *et al.* 2001). Similar genes have also been observed in the platyfish *Xiphophorus maculatus* (Orzack *et al.* 1980) and in the rice fish *Oryzias latipes* (Wada *et al.* 1998). The evidence for the link of color genes and sex modifiers in *N. omnicaeruleus* is given below.

Breeding experiments in the laboratory showed that WB and OB fish homozygous for the gene that codes for WB or OB are almost black or orange, respectively, while heterozygous fish, obtained from crosses with P fish, have blotched phenotypes with much more white (WB) or black (OB). Crossings between heterozygous WB females and heterozygous WB males showed that the WB gene is sex linked (Table 8.2). The same was found in crossings between heterozygous OB females and heterozygous OB males. Crosses between WB females and OB males yielded no homozygous blotched offspring, which suggests that WB and OB are alleles on different loci. The occurrence of intermediate phenotypes between blotched and plain in crosses between blotched and some plain individuals is consistent with the presence of a locus (M, m) with additive effects on the intensity of expression of WB and OB genes. Thus, the minimum model that can explain the observed color segregation is a three-locus, six-allele model. Two alleles at each of two sex-linked loci determine whether a phenotype is “plain” (aabb), WB (Aabb, AAbb), or OB (aaBb, aaBB). At the modifier locus, M results in a higher intensity of expression of the blotch gene than m.

The absence of males (homozygous for blotched) and the observed recombination fraction ($r = 0.052$) obtained in crosses with blotched males (heterozygous for color and sex) suggest that both blotch loci are X-linked. The third locus (m, M) appears to be autosomal, because crosses indicate that both sexes can be homozygous for either allele and because the recombination fractions ($0.4 < r < 0.6$) between the M locus and the sex chromosome are high.

When at least one parent carried the allele m at the modifier locus, crosses expected to yield blotched males (e.g., males of a P line mated with blotched females) actually produced significantly skewed sex ratios. Two crosses of homozygous WB females with P males yielded all female clutches. Crosses with heterozygous OB females and P males yielded significantly fewer OB males than expected on

Table 8.2 Results of breeding experiments. First column and first row give the parental phenotypes. Allele A represents the blotch allele at both the WB and the OB locus, which show parallel inheritance, allele M represents M_A and M_B (see text). P, plain morph; B, blotched morphs (a) through (d) represent degrees of blotchiness as affected by the modifier locus with alleles M, m. Mate preferences of all WB-line individuals and all P-line individuals crossed with them were tested prior to the breeding experiments. Observed and expected phenotypic segregations are given as a percentage in each box. The sex effects of the X-linked female determiner and the autosomal male determiner are incorporated. The probability for the B(d) phenotype in box 6 is the sum of all the probabilities for the AAMmxx and the AAMMxx genotypes, which cannot be distinguished visually. Deviations from expectations are tested by χ^2 for color and sex separately, and for the color–sex combination using the recombination fraction of 0.052.

Father/ Mother	Blotched AaMMxy		P of blotched aaMMxy		P of P-line aammxy				
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.			
Blotched (d) AAMMxx	B(d)	41.50	47.50	B(c)	44.90	50.00	B(b)	100	100
	B(c)	4.70	2.50						
	B(d)	–	2.50	B(c)	55.10	50.00			
	B(c)	54.70	47.50						
	Color $\chi^2=1.53, df=1, p=0.22$		Color $\chi^2=0df, p=1$		Color $\chi^2=0, df, p=1$				
	Sex $\chi^2=0.47, df=1, p=0.69$		Sex $\chi^2=0.51, df=1, p=0.48$		Sex $\chi^2=0, df, p=1$				
	Comb $\chi^2=0.44, df=3, p=0.44$		Box 1		Box 2		Box 3		
Blotched (c) AaMMxx	B(d)	23.00	23.70				B(b)	39.50	48.70
	B(c)	25.50	23.70				P	28.90	25.00
	P	0.60	2.60						
	B(d)	–	2.60				B(b)	5.30	1.30
	B(c)	26.70	23.70				P	26.30	25.00
	P	24.20	23.70				Color $\chi^2=0.42, df=1, p=0.52$		
	Color $\chi^2=1.14, df=2, p=0.56$		Color $\chi^2=0.16, df=1, p=0.69$		Color $\chi^2=0.54, df=1, p=0.46$		Comb $\chi^2=1.30, df=2, p=0.52$		
Sex $\chi^2=0.16, df=1, p=0.69$		Comb $\chi^2=0.44, df=5, p=0.19$		Box 4		Box 5			
Blotched (d) AaMmxx	B(d)	21.40	23.70				B(b)	12.50	24.35
	B(c)	21.40	11.85				B(a)	12.50	25.00
	B(b)	17.90	23.08				P	12.50	25.00
	P	3.60	2.60						
	B(d)	–	2.60				B(b)	6.30	0.65
	B(c)	17.90	11.85				P	56.20	25.00
	B(b)	–	0.62				Color $\chi^2=3.14, df=3, p=0.37$		
Color $\chi^2=3.14, df=3, p=0.37$		Color $\chi^2=0.12, df=1, p=0.73$		Color $\chi^2=0.12, df=1, p=0.73$		Sex $\chi^2=0.12, df=1, p=0.73$			
Sex $\chi^2=0.12, df=1, p=0.73$		Comb $\chi^2=4.78, df=6, p=0.57$		Comb $\chi^2=4.78, df=6, p=0.57$		Box 6			
P aammxx	B(b)	50.90	47.50	P	28.20	50.00	P	54.90	50.00
	P	3.50	2.50						
	B(b)	5.30	2.50	0					
	P	40.40	47.50	P	71.80	50.00	P	45.10	50.00
	Color $\chi^2=0.86, df=1, p=0.35$		Color $\chi^2=0, df, p=1$		Color $\chi^2=0, df, p=1$		Color $\chi^2=0, df, p=1$		
	Sex $\chi^2=0.44, df=1, p=0.51$		Sex $\chi^2=13.50, df=1, p=<0.05$		Sex $\chi^2=1.38, df=1, p=0.24$		Sex $\chi^2=1.38, df=1, p=0.24$		
	Comb $\chi^2=1.77, df=3, p=0.62$		Box 7		Box 8		Box 9		
Box 8		Box 9		Box 10		Box 10			

the basis of a 0.5 Fisherian sex ratio. This can be explained by a female determining gene (W), tightly linked to both X and a blotched color allele, of which the dominance over Y is complete in WB, but not in OB. Both P males from blotched parents and blotched males when crossed with blotched females produce normal sex ratios, which do not deviate from 0.5. Hence, these must differ genetically from males of the P line. Males from blotched parents must not only possess a gene that enhances blotch expression in their blotched heterozygous offspring, but also a recessive male-rescue gene that counteracts the sex effects of W. These maleness effects and color effects are associated and might be caused by the same locus (Table 8.2).

Phenotype frequencies in nature

A total of 2600 adult specimens were collected between 1990 and 1996 at Makobe island in Lake Victoria. The frequencies of the ten observed phenotypes differ strikingly: P, WB, and OB females and P males are much more abundant than are the others. In particular, it is striking that WB and OB males are extremely rare in nature (Figure 8.2). Note that many genotypes that can easily be obtained in laboratory breeding are lacking in nature, because mating is not random. The latter has been shown in mate-choice experiments.

Mate-choice experiments

The difference between P males of a true breeding plain population P_P and P males that originate from blotched parents (P_{WB} and P_{OB}), as discovered in the experiments on the genetics of coloration, was also reflected in their mate-choice behavior. In female choice/male no-choice trials WB females were courted more often by P_{WB} than by P_P males, and more often by WB males than by P_P males. The WB females were equally responsive to the three kinds of males. P_P females were courted as often by P_P males as by P_{WB} males. They were also courted equally often by WB and P males, but responded to and followed P males more often than WB males. Hence, independent of male courtship activity, P females preferred P males, but WB females did not exhibit preferences. In a no-choice situation, P_P was the only choosy male type. In choice experiments, P_P males also ignored WB females. WB males courted WB females more often than P females. P males from P parents courted P females more often, but P males from WB parents behaved as WB males and courted WB females more often (Figure 8.3).

Summarizing the evidence

Thus, Seehausen *et al.* (1999) have shown that differences at possibly as few as three loci give rise to conspicuous and, in nature, discrete color variation, with three common morphs: “plain”, “white-and-black blotched”, and “orange blotched”. Genetics alone does not explain the relative frequencies of the different morphs in nature. The combination of a high frequency of blotched females with a low frequency of the M allele at the modifier locus in plain morphs in nature is incompatible with random mating. Intermediate phenotypes were distinctly

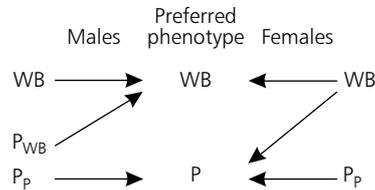


Figure 8.3 Summary of male and female mating preferences in *N. omnicaueruleus*. One arrow indicates a mating preference for either plain colored or white-and-black blotched partners. Two arrows indicate that no preference is exhibited for the two types of partners.

less abundant than expected under random mating. The evidence from the mate-choice experiments is in agreement with the field evidence that mating between color morphs is nonrandom. A sex-determining gene linked to color can explain the evolution of preferences. Mating preferences in color morphs of *N. omnicaueruleus* might have evolved by sex-ratio selection to avoid matings that result in progeny with skewed sex ratios or to avoid fitness loss through the production of fewer fit YY-homozygotes. For complete interruption of the gene flow between the blotched and plain morphs, preference of blotched females for blotched males may be required. However, the rarity of blotched males in nature selects against the spread of a gene for such a preference and prevents full speciation.

Modeling speciation in haplochromines with sex reversal

Recent models by Lande *et al.* (2001), based on the genetics and the mate-choice behavior summarized above, show that the combination of sexual selection by mate choice and sex-ratio selection provides a pathway for sympatric speciation. The model considers mate choice by both sexes, as observed by Seehausen *et al.* (1999). Novel color genes can invade against sexual selection because they are initially largely restricted to females (as the male-rescue gene is rare). This causes sexual selection on mating preferences in males for the new female colors. Once the new preference is sufficiently frequent, it, in turn, causes sexual selection by female choice on the initially rare males with new color (and male-rescue genes), leading to sudden complete speciation. The evolution of a novel mating preference in the model is driven by the spread of a dominant sex-determining gene W. This gene can spread in the population when it has some intrinsic selective advantage. This would be so when either the female-biased sex ratio that W produces is selected for by intrademic group selection, or when W has a pleiotropic effect that increases reproductive success to compensate for selection against female-biased sex ratios in panmictic populations. Matings between XY males and WY females will result in YY zygotes.

If YY males are not viable, because of deleterious Y-linked mutations, the establishment of the W gene, followed by the spread of a W-linked new color mutation can result in the above-described sympatric speciation scenario: preference for the new color evolves and a dominant female determiner W together with a recessive suppressor gene M spread to fixation. This suppressor counteracts the

effects of W in animals homozygous for M, overcomes the production of inviable YY individuals, and restores the sex ratio.

If YY males are viable, the establishment of the W gene results in a change in the heterogametic sex (XX females and XY males in the parental population, YY males and WY females in the derived population). This is in accordance with the observation that species of the tilapiine cichlid genus *Oreochromis* often differ in the heterogametic sex.

Lande *et al.*'s model explains X-linked sex-reversal, often observed in cichlids, and the strong association between sex reversal and color, as found for the blotch polymorphism in *N. omnicaeruleus* and other haplochromine cichlids.

The model also shows that sex-ratio selection, together with sexual selection for color, can result in sympatric speciation. Full speciation was not found in the empirical study of *N. omnicaeruleus*, possibly because the rarity of blotched males in nature prevents the evolution of preference by blotched females for males of their own kind. The rarity of these males, in contrast to the high frequency of blotched females, can be explained if two opposed selection forces act on the polymorphism. Given that blotched males are produced at normal ratios when blotched females mate with males of the MM genotype, we have to invoke natural selection against them to explain their rarity. Territorial males that perform conspicuous displays to attract females must expose themselves more than females to the abundant avian predators. This natural selection against blotched males might then be balanced by sexual selection.

Last but not least, Lande *et al.*'s model helps to explain why rock-dwelling haplochromines have undergone more speciation than other cichlids. Rock-dwelling cichlids are territorial habitat specialists with little dispersal. Hence, populations in rocky habitat patches often experience high degrees of genetic isolation (van Oppen *et al.* 1997; Arnegard *et al.* 1999). Such spatially structured populations are more likely to accumulate recessive, deleterious Y-linked mutations that result in low YY-male fitness.

At present we do not know what constitutes the intrinsic selection advantage that allows the W gene to spread initially. The fitness of YY-homozygotes is a subject of ongoing research.

Field evidence for sympatric speciation in haplochromine cichlids

In nature, closely related species are isolated neither by the timing of reproduction nor by the location of reproduction (Seehausen *et al.* 1998). This makes it most likely that direct mate choice is the factor that keeps closely related species genetically isolated and maintains species diversity in the absence of postzygotic barriers to hybridization. Compelling evidence for this is given in the study by Seehausen *et al.* (1997) reviewed above.

Based on the hypothesis that sympatric speciation, caused by disruptive sexual selection, contributed to the origin of haplochromine species diversity, is a prediction that the proportion of pairs of closely related species that are different colors is higher among sympatric pairs than among allopatric pairs. An analysis of patterns

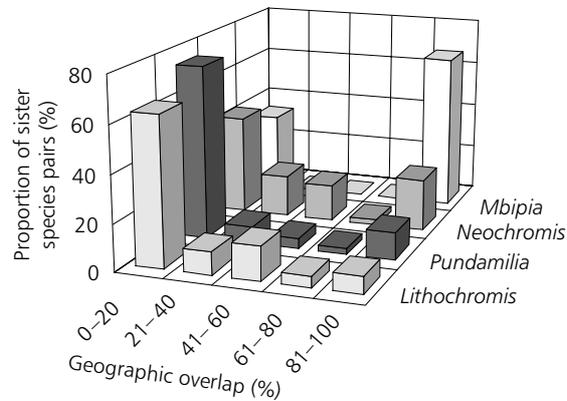


Figure 8.4 The proportions of congeneric species pairs and the numbers of congeneric species pairs plotted against geographic distribution overlap, for four genera of rock-dwelling Lake Victoria haplochromines.

of geographic distribution of 41 species supported this prediction. In particular, strong support for sympatric speciation came from yellow–red versus blue species pairs, and less strong evidence came from plain versus blotched species pairs in the genus *Neochromis* (Figure 8.4; Seehausen and van Alphen 1999, 2000). Extra evidence for sympatric speciation in Lake Victoria cichlids comes from the form of the frequency distribution of the proportion of geographic overlap in the distribution of closely related species. Without sympatric speciation, this distribution would be expected to have a single hump. However, the proportion of geographic overlap between closely related species shows a bimodal frequency distribution, with peaks at both the fully allopatric and fully sympatric positions.

8.5 Pharyngeal Jaw Versatility and Feeding Diversification

Survival of a new form depends on its ability to respond to ecological demands. Here, the versatile anatomy of cichlids, their phenotypic plasticity, and their capacity to adapt their behavior through learning come into play. Here, the greater versatility of the cichlid anatomy over that of their percoid ancestors is shown.

Ecological diversification

The genetic isolation of subpopulations by sexual selection, as described for haplochromines, is nonadaptive, because it results in ecological homologs. Disruptive natural selection is needed for the divergence of young species. The slight, but significant and adaptive, ecological differences that are often observed between closely related species may evolve under disruptive natural selection, which can easily operate after the termination of gene flow by assortative mate choice for males with different body coloration. For lasting coexistence, ultimately both mate choice and natural selection may need to be disruptive (Lande and Kirkpatrick 1988).

Most cichlids are opportunistic feeders, and all feed on nutrient-rich food when it is available. Yet, there is ample evidence for ecological radiation in the haplochromines. Species have diversified into specialists on detritus, aufwuchs, zooplankton, phytoplankton, insects, fish, fish scales, fish parasites, gastropods, and so on. Species are further segregated by depth in the water column, by habitat, and by behavior (e.g., different modes are used to eat algae from the rocks). This enables a very large assemblage of coexisting species. Although Lake Victoria haplochromines most likely evolved from a single ancestor over a short evolutionary time scale, they represent an impressive array of ecological adaptations.

Pharyngeal jaw apparatus in cichlids and other labroids

The cichlids belong to the labroid fishes (Perciformes), an extensively diversified group. This diversification has been attributed to the development of a morphologically specialized and functionally versatile pharyngeal jaw apparatus (Fryer and Iles 1972; Greenwood 1974; Liem 1973, 1978, 1979; Yamaoka 1978; Liem and Sanderson 1986; Jensen 1990). The structure and function of the derived pharyngeal jaw apparatus of labroids has been studied extensively (Greenwood 1965; Liem 1973, 1978, 1979; Yamaoka 1978; Hoogerhoud and Barel 1978; Liem and Greenwood 1981; Kaufman and Liem 1982; Liem and Sanderson 1986; Stiassny and Jensen 1987; Galis 1992, 1993a, 1993b; Vandewalle *et al.* 1992), in contrast to that of nonlabroid Perciformes, which is less derived and retains more ancestral conditions [Liem (1970) for Nandidae, Lauder (1983a, 1983b) for Centrarchidae, Wainwright (1989) for Haemulidae, and Vandewalle *et al.* (1992) for Serranidae].

Liem (1973) suggested that the exceptional speciosity of cichlids is a consequence of an evolutionary innovation: the versatile pharyngeal apparatus. Recent support for Liem's hypothesis is provided by a comparison of the pharyngeal jaw apparatus in cichlid fishes with that of their presumed generalized percoid ancestors (Galis and Drucker 1996). The results of this comparison suggest support for a more general hypothesis by Vermeij (1974), namely that speciose taxa are characterized by more independent elements than taxa that are less speciose. A large number of independent elements increase the number of potential solutions for a particular biomechanical problem. Therefore, body plans with more independent elements can be modified and diversified more easily than those with fewer independent elements.

Galis and Drucker (1996) compared the mechanics of the pharyngeal biting of the centrarchids (a less-derived percoid family) with those of cichlids, by developing a mechanical model of pharyngeal biting in centrarchids. This was based on anatomic observations and manipulations in live fishes. The centrarchid model was tested by electrically stimulating individual muscles and observing the resultant movements.

Similar muscle stimulation experiments were carried out with the cichlid fish *Labrochromis ismaeli*, while anatomic studies were carried out with the cichlids *O. niloticus*, *Cichlasoma citrinellum*, and *L. ismaeli*.

The anatomic research revealed that there are three important structural couplings among the elements of the pharyngeal jaw apparatus of centrarchids (Figure 8.5). Two of these are modified in the cichlids:

1. *Fourth epibranchials – upper pharyngeal jaws.* In the centrarchids, there is an articulation between the lateral face of the upper pharyngeal jaw and the medial surface of epibranchial 4, as has been found in heamulids (Wainwright 1989). This coupling transmits the force exerted by the m. levator posterior and externus 4 on epibranchials 4 to the upper pharyngeal jaws. In the cichlids this coupling is lost. The fourth epibranchials lie against the surface of the upper pharyngeal, but the two elements are capable of independent movement. Unlike in the centrarchids, the m. levator externus of the cichlids runs from the neurocranium to the lower pharyngeal jaws, rather than to epibranchial 4, and it suspends the fused lower pharyngeal jaws in a muscle sling. The force of this muscle therefore acts directly upon the lower pharyngeal jaw, instead of indirectly upon the upper pharyngeal jaw via rotation of the fourth epibranchials.
2. *Fourth ceratobranchials – lower pharyngeal jaws.* In the Centrarchidae the lower pharyngeal jaws are firmly connected to the fourth epibranchials. This greatly restricts independent movement of the lower pharyngeal jaws and the fourth ceratobranchials. In the Cichlidae, this coupling is replaced by a more flexible connection, which permits movement of the lower pharyngeal jaws with respect to the ceratobranchials.
3. *Fourth epibranchials – lower pharyngeal jaw(s).* In the centrarchids, there is a strong connection between the fourth epibranchials and the lower pharyngeal jaws, which is both ligamentous and muscular. This direct connection strengthens the second coupling in linking the movements of the fourth epibranchials and the lower pharyngeal jaws. The third coupling has been retained in cichlids.

The anatomic model and its test by electrostimulation of muscles showed that these couplings unite the movements of the upper and lower pharyngeal jaws in centrarchids, and therefore seriously constrain the number of biting movements and, thus, the number of activity patterns of the pharyngeal jaw muscles. In contrast, loss of the first and second couplings in cichlids resulted in independent movement of the upper and lower pharyngeal jaws. This allows the generation of biting forces in many different directions (Galis 1992). In addition to this, the lower pharyngeal jaws in cichlids are fused and a strong bond exists between the upper pharyngeal jaws. This provides a twofold increase in the force that can be applied to the prey (Figures 8.5a, 8.5b, and 8.5c). Thus, the functional flexibility of cichlids is enhanced in comparison with that of more primitive labroid fish like the centrarchids (Figure 8.6). This increase in versatility of the pharyngeal allows efficient handling of many different prey items (Liem 1973; Stiassny and Jensen 1987; Galis and Drucker 1996) and the resultant flexibility allows improved phenotypic response to changing requirements. Like other forms of phenotypic plasticity, it may be genetically assimilated subsequently (Galis and Metz 1998). This decreases the probability of extinction after colonization of a new environment and increases the

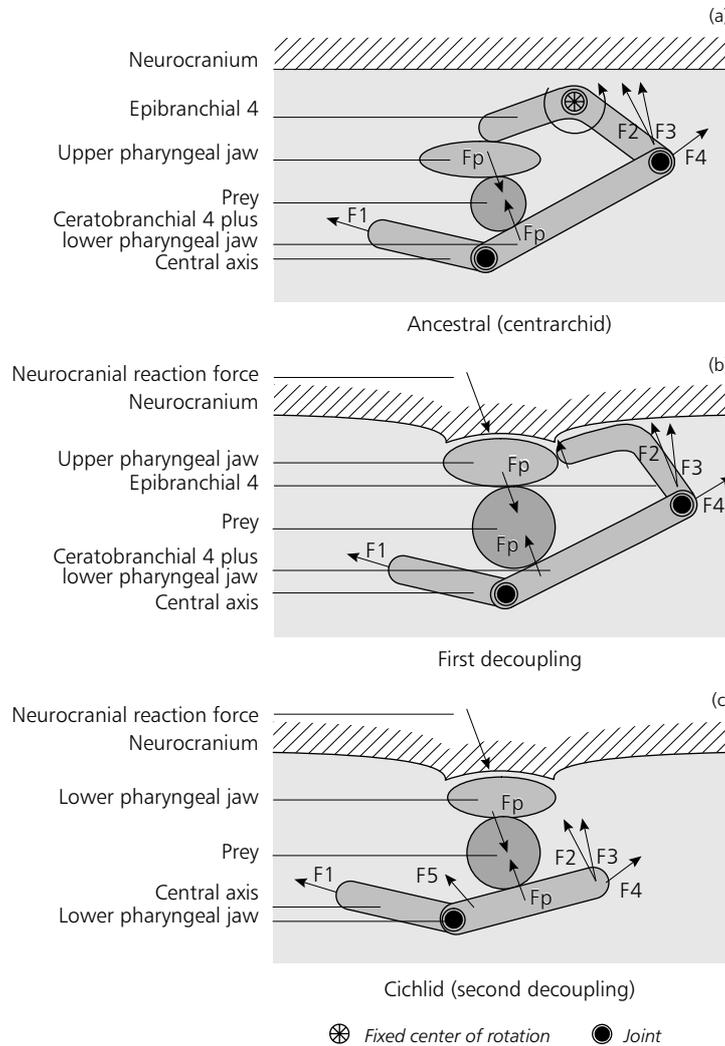


Figure 8.5 Representations of biting with pharyngeal jaws. (a) Presumed centrarchid-like ancestors of cichlids (coupled movement of the upper and lower pharyngeal jaw is caused by F1 and F2; rotation of epibranchial 4, part of the fourth gill arch, causes the upper pharyngeal jaw to move down). (b) Fishes with the hypothesized intermediary state after the decoupling of the upper and lower pharyngeal jaw (there is no rotation and, thus, the upper pharyngeal jaw is not pushed down, but pushed up against the neurocranium; the fourth gill arch and the lower pharyngeal jaw are together pulled up by F1 and F2). (c) Cichlids after the decoupling of the lower pharyngeal jaw and the fourth gill arch (ligamentous and muscular connections have disappeared between the lower pharyngeal jaw and the fourth gill arch and the muscles that produce F1 and F2 are inserting directly on the lower pharyngeal jaw instead of on epibranchial 4).

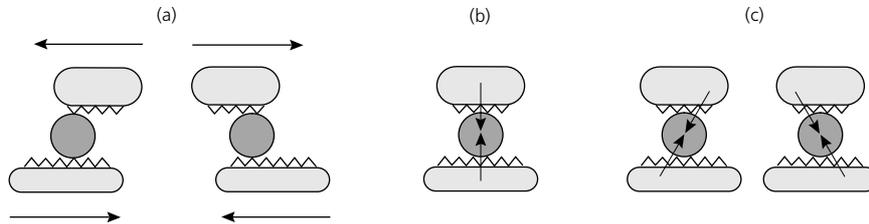


Figure 8.6 Possibilities of movement of the pharyngeal jaw apparatus of a centrachid-like ancestor of cichlids (a or b) and of a cichlid (a and b and c). Note the much increased versatility of the pharyngeal jaw apparatus after the two decouplings. Sources: Galis (1992) and Galis and Drucker (1996).

likelihood of long-term population survival. The enhanced functional flexibility of cichlids not only provides phenotypic plasticity, but also means that only small evolutionary changes are necessary to change feeding specialization. In addition to the versatility of the pharyngeal jaws, the cichlid design enables rapid adaptation to new ecological circumstances by changes in allometric growths of structures in the head (Greenwood 1974, 1981, 1994). Such changes could possibly be induced by changes in the timing during development, and could be coded for by only a few genes (Gould 1977). Such changes can also be induced phenotypically, as juveniles grow up. This phenotypic plasticity is important for the survival of individuals that have to adapt to a new environment (Galis and Metz 1998; Bouton *et al.* 1999).

8.6 Concluding Comments

The unusually fast ecological radiation of haplochromine cichlids and the exceptionally dense species packing in several lineages of African cichlids raises the question of what is different in these cichlids. Most lacustrine species flocks of other fish taxa, even other cichlid taxa, are less diverse in ecology and species numbers. The versatility of the pharyngeal jaw apparatus, physiological properties, and mouth-brooding behavior may all be necessary attributes, but these alone are not sufficient to explain the exceptionally fast radiation of haplochromines. The evidence presented above shows that the combination of a number of factors are needed to explain such radiation. The polygynous breeding system of haplochromines is associated with strong asymmetric investment in parental care, in which the females invest much more than males. Such a breeding system is conducive to sexual selection on male characters. It has resulted in the sexually dimorphic breeding coloration, with brightly colored males and often dull females. If water transparency allows, disruptive sexual selection on male coloration can result in genetic isolation between fish that differ in male coloration and female preference for male coloration. Disruptive sexual selection can act either in full sympatry, or reinforce genetic isolation of populations that have developed different male coloration in allopatry and have become sympatric secondarily by range expansion. The relative contribution of sympatric and allopatric speciation to the

haplochromine species flocks is still unclear. A premating barrier between populations with differently colored males alone is not sufficient to allow long-term coexistence of these populations. However, such a premating barrier prevents the homogenizing effect of recombination in a contiguous population. This facilitates the build-up of ecological differences between the populations by disruptive natural selection. Ecological differences between closely related haplochromines comprise their microdistribution and/or small functional differences in the feeding apparatus (Seehausen and Bouton 1997). Such differences may evolve fast because of the versatile cichlid jaw apparatus and because of the possibly simple genetics that underlie allometric changes of the feeding apparatus.

One additional factor that might drive rapid speciation is a dominant sex-reversal gene that results in sex-ratio selection acting in concert with sexual selection for both male and female coloration. Finally, some aspects of the reproductive biology of haplochromines are important. Haplochromines have parental care by mouth brooding, which frees them from reproductive constraints experienced by free-spawning fish. Such constraints can result from insufficient oxygen supply for eggs and larvae and/or the necessity to migrate to shallow water or rivers for spawning. The mouth-brooding behavior of haplochromines makes them relatively independent of the spawning substrate (Fryer and Iles 1972), which can be a scarce commodity on the soft muddy bottoms that prevail at greater depths in lakes.

Other fish have formed species flocks in sympatry, such as the Lake Tana barbs (Nagelkerke and Sibbing 1996) and the tilapiine cichlid species from Lake Barombi-Mbo (Schliewen *et al.* 1994). Yet, the haplochromines of Lakes Malawi and Victoria stand out by their species numbers (one or two orders of magnitude more than the flocks in Lakes Tana and Barombi-Mbo). The haplochromine flocks also stand out in comparison with other tilapiine cichlids, co-occurring with them in Lakes Malawi and Victoria. Why have these *Oreochromis* species not radiated into large species flocks? They share almost all the attributes that make the haplochromines prone to rapid radiation. This suggests that something present in the genetic make-up of haplochromines, but absent in *Oreochromis*, predisposes haplochromines to rapid speciation. Possibly this is the presence of a color-linked sex-reversal gene, as found in the blotched morphs of haplochromines; possibly it is a genetic mechanism that allows species repeatedly to split into red-yellow and blue species pairs without exhausting the genetic variation for coloration in the daughter species. Detailed genetic studies are needed to solve this riddle. This is yet another reason to continue the study of haplochromines as a model for speciation. The lack of a postmating barrier in these fish, the high rate of speciation, and many of their other biological attributes allow the processes that may operate in a wide variety of sexually dimorphic animals to be studied with relative ease.

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