

The diversity of male nuptial coloration leads to species diversity in Lake Victoria cichlids

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The amazing coloration shown by diverse cichlid fish not only fascinates aquarium keepers, but also receives great attention from biologists interested in speciation because of its recently-revealed role in their adaptive radiation in an African lake. We review the important role of coloration in the speciation and adaptive evolution of Lake Victoria cichlids, which have experienced adaptive radiation during a very short evolutionary period. Mature male cichlids display their colors during mate choice. The color of their skin reflects light, and the reflected light forms a color signal that is received by the visual system of females. The adaptive divergence of visual perceptions shapes and diverges colorations, to match the adapted visual perceptions. The divergence of visual perception and coloration indicates that the divergence of color signals causes reproductive isolation between species, and this process leads to speciation. Differences in color signals among coexisting species act to maintain reproductive isolation by preventing hybridization. Thus, the diversity of coloration has caused speciation and has maintained species diversity in Lake Victoria cichlids.

Key words: color signals, Lake Victoria cichlid, male nuptial coloration, opsin, species diversity

INTRODUCTION

Understanding the mechanisms underlying the generation and maintenance of biodiversity is a central issue in evolutionary biology. Biodiversity is thought to be generated through the repetition of speciation events over evolutionary time. In speciation, one ancestral population is divided into two or more populations, and reproductive isolation is established between the populations (Coyne and Orr, 2004).

One of the driving forces generating such reproductive isolation is the divergence of mating signals: individuals of one sex prefer signals with certain traits from the other sex in their choice of mates. Divergence of mating signals has long been thought to play a key role in the establishment of premating reproductive isolation and in the maintenance of coexisting closely-related species (Endler, 1992; Boughman, 2002; Rundle et al., 2005; Miyagi et al., 2012). The reflection of light by mating traits creates a color signal that is received by visual perception. Color signals are used as mating signals, and colorful male

ornaments are diversified between closely-related species in several animals. Examples are found in the coloration of fishes, the pelage coloration of birds, the dewlap coloration of anolis lizards, and the wing coloration of butterflies (Boughman, 2001; Jiggins et al., 2001; Roulin, 2004; Seehausen et al., 2008; Losos, 2009).

One well-known case of color signal divergence is found in cichlid fish (Terai and Okada, 2011). The coloration of male Lake Victoria cichlids is diversified between closely-related species (e.g., in nuptial coloration, as shown in Fig. 1) (e.g., Seehausen, 1996), and sexual selection via female choice of male coloration has played an important role in the evolution of Lake Victoria cichlids (Seehausen et al., 1997a, 2008; Maan et al., 2006). Model simulations propounded the theory that local adaptations of the visual system to different light environments caused divergence of color via signal matching to the adapted perception of mates (Kawata et al., 2007). Recently, analyses of male color and vision in cichlids revealed that color signal divergence leads to speciation in nature, supporting this model (Seehausen et al., 2008). In this review, we summarize recent studies of color signal divergence, and explain its role in speciation and in the maintenance of species diversity in Lake Victoria cichlids.

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Lake Victoria cichlids Cichlids, freshwater fishes from the family Cichlidae, form the most species-rich family of vertebrates. More than 3,000 species of cichlids exist in Central and South America, Africa, Madagascar and Southern India (Kocher, 2004). Rapid adaptive radiation of cichlid species in the Great Lakes of East Africa (Lakes Victoria, Tanganyika and Malawi) is an attractive example of vertebrate evolution for evolutionary biologists (Fryer and Iles, 1972; Seehausen, 1996; Turner et al., 2001; Kocher, 2004), and African cichlids form an ideal model system for understanding the genetic basis of vertebrate speciation (Kornfield and Smith, 2000; Kocher, 2004). Lake Victoria cichlids in particular have experienced very rapid radiation, and the lake now harbors a unique species-rich community of more than 500 endemic cichlid fish species (Seehausen et al., 1997b). Lake Victoria has dried up and has been refilled several times in its history. The latest such cycle is estimated to have taken place approximately 15,000 years ago (Johnson et al., 1996, 2000). Thus, the present cichlid species are thought to have experienced explosive adaptive radiation during a very short evolutionary period (Meyer et al., 1990; Nagl et al., 2000; Seehausen, 2002; Verheyen et al.,

2003; Elmer et al., 2009).

Since Lake Victoria cichlids are a very young species group, their genetic diversification is extremely low (Nagl et al., 1998, 2000; Terai et al., 2004). Because of low genetic differentiation among species and due to a lack of post-zygotic reproductive isolation, Lake Victoria cichlid species could potentially interbreed with fertility (Seehausen et al., 1997a), if interspecific mate choice did not take place (Crapon de Caprona and Fritzsche, 1983; Crapon de Caprona, 1986). In nature, instead of post-zygotic reproductive isolation, pre-zygotic reproductive isolation through mate choice by color signals is thought to eliminate gene flow between species.

Lake Victoria cichlids inhabit various environments such as rocky and sandy shores, offshore deep water, and shallow vegetation-covered shores (Witte and van Oijen, 1990; Seehausen, 1996). Their behavior and morphology are adapted to their habitats. Therefore Lake Victoria cichlid species are usually defined by their morphologies. Male color is one of the most diversified morphologies (see for example the divergence of nuptial colorations shown in Fig. 1) and used as an important categorical trait in Lake Victoria cichlid classification (e.g., Witte and van

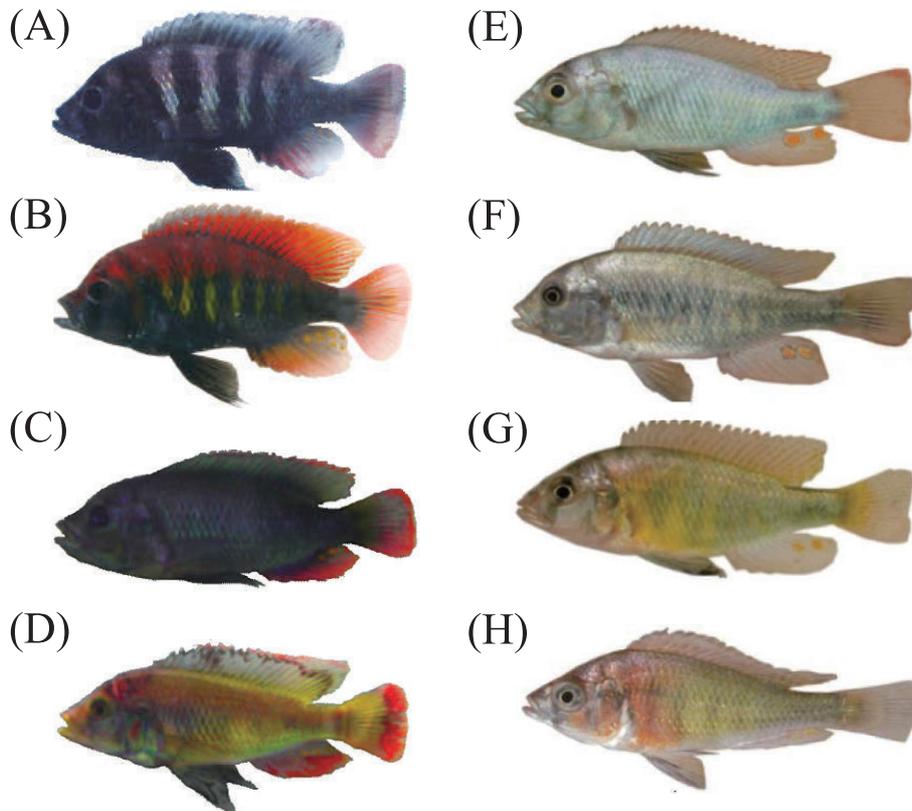


Fig. 1. Male nuptial coloration in Lake Victoria cichlids. Photographs of male nuptial colors in eight Lake Victoria cichlid species. Photographs of (A) *P. pundamilia* and (B) *P. nyererei* are modified from Seehausen et al. (2008). Photographs of (C) *N. greenwoodi* and (D) *N. greenwoodi* are modified from Terai et al. (2006). Photographs of (E) *Platytaniodus degeni*, (F) *Haplochromis fischeri*, (G) *Haplochromis* sp. 'thick skin', and (H) *Haplochromis* sp. 'macula' are modified from Miyagi et al. (2012).

Oijen, 1990; Seehausen, 1996). As mentioned above, Lake Victoria cichlids are genetically closely related and could potentially interbreed, although several species in which males differ in color are sympatric (Seehausen et al., 1997a; Miyagi et al., 2012).

Cichlid color The colorations of fish are multilayer, multicomponent signals (different color pigment cells in different layers) similar to those in reptiles and amphibians (Grether et al., 2004). The colors seen in the skin of fish are due to the presence of pigment cells called chromatophores, comprising light-absorbing melanophores, xanthophores and erythrophores, and light-reflecting iridophores (Fujii, 1993). Melanophores are black and contain black melanin pigment synthesized from tyrosine (Bagnara and Hadley, 1973). Xanthophores (yellow) and erythrophores (red) contain carotenoid- and pteridine-derived pigments, which act as color filters, preferentially absorbing light of short wavelengths (Bagnara and Hadley, 1973). Iridophores, which mainly contain reflecting crystalline platelets of guanine, are iridescent or silvery and produce structural coloration (Fujii, 1993, 2000). In combination, the absorbance and reflectance of particular wavelengths of light by these chromatophores generates specific fish colorations. The colors of cichlids are also generated by combinations of these chromatophores. For example, the yellow spots that mimic the eggs of cichlids, called “egg dummies” (see the yellow spots on the anal fins in Fig. 1), are made up of high densities of xanthophores (Salzburger et al., 2007).

Mature males of Lake Victoria cichlids have bright colors on their lateral bodies, fins and heads, and some vertical black bars (Fig. 1) (e.g., Seehausen, 1996), known as nuptial coloration. By contrast, female cichlids show pale colors, such as metallic gray and pale yellow (e.g., Seehausen, 1996). The colors of Lake Victoria cichlids are heritable (Seehausen et al., 1997a; van Der Sluijs et al., 2008), and the numbers of genes involved in the formation of colors have been estimated by crossing closely-related species. *Pundamilia pundamilia*, which has blue-gray nuptial coloration (Fig. 1A) made by iridophores, and *P. nyererei*, which has yellow-red nuptial coloration (Fig. 1B) made by xanthophores and erythrophores, were crossed, and the minimum numbers of genes controlling these colorations were estimated. Red and yellow colorations may be controlled by 2–4 and a single gene, respectively (Magalhaes and Seehausen, 2010). Examples of candidate genes that may control nuptial coloration in cichlids are as follows: *hagoromo*, which is involved in the formation of the colored stripe pattern in zebrafish (Terai et al., 2002b); *csf1ra*, which is expressed in xanthophores in the egg dummies of cichlids (Salzburger et al., 2007); and *c-ski1* and *pax7*, which are tightly linked to the color pattern of orange blotches (Streelman et al., 2003; Roberts et al., 2009). However detailed roles of

these candidate genes in cichlid pigmentation are largely unknown. Various types of pigmentation variations are observed in many animal species and the genetic factors generating such variations are identified in several organisms such as flies (Takahashi, 2013) and mammals (Suzuki, 2013). The information of the genes responsible for pigmentation variations in other animals may provide new insights into molecular mechanisms of the formation of male nuptial coloration in cichlids.

Roles of cichlid nuptial coloration As mentioned previously, mate choice plays an important role in their reproductive isolation. How do females choose their mating partners? Male nuptial coloration generally represents the male’s condition, such as his position within the social hierarchy and his health. Healthy and dominant males show bright nuptial coloration, whereas weaker males are less bright. Females select males with bright nuptial colors as mating partners; such males may produce more offspring. Mate choice experiments using two closely-related *Pundamilia* species, *P. pundamilia* (Fig. 1A, blue-gray nuptial coloration) and *P. nyererei* (Fig. 1B, red nuptial coloration), showed that under white light, color discrimination was available and females preferred the nuptial coloration of conspecific males. Under monochromatic light, color discrimination was unavailable and females preferred big males or those with high display rates (Seehausen and van Alphen, 1998). These experiments revealed that females prefer the nuptial coloration of conspecific males (Seehausen and van Alphen, 1998; Stelkens et al., 2008). Females of *P. nyererei* in particular also preferred more conspicuously-colored males (Maan et al., 2004).

The perception of conspicuousness is influenced by ambient and background light, signal transmission, receiver sensitivity and higher-level processing (Endler, 1993; Boughman, 2002). Here, we focused on the receiver sensitivity and ambient light of two *Pundamilia* species. Females of *Pundamilia* species have heritable preferences and species-specific sensitivities to different light wavelengths (Haesler and Seehausen, 2005; Maan et al., 2006). *P. nyererei* (red coloration) has a low threshold for the detection of red light (high sensitivity to red light), whereas *P. pundamilia* (blue coloration) has a low threshold for the detection of blue light (high sensitivity to blue light) (Maan et al., 2006). In *P. nyererei*, females have stronger preferences for brighter male coloration (Maan et al., 2010). These colorations and preferences are associated with the ambient light conditions, blue- and red-dominated, found in the habitat of each species, respectively. Hence, male colorations of both species create stronger signals for female sensitivities by reflecting ambient lights in their habitats, respectively.

Males use their coloration not only as a mating signal but also in male–male competition. In *Pundamilia* spe-

cies, males recognize the color of conspecific males and use this information in social behavior such as threat behavior to the other males (Dijkstra et al., 2006, 2010). Male–male competition is regarded as a significant force in the maintenance of phenotypic diversity (Dijkstra et al., 2009). Male–male competition and nuptial color displacement also lead to diversifying forces for species (Seehausen and Schluter, 2004).

Cichlid vision Cichlids perceive color signals by color vision, as do other animals. Color vision in Lake Victoria cichlids has been the subject of behavioral studies (Seehausen and van Alphen, 1998; Maan et al., 2006), histological studies (van der Meer and Anker, 1983; van der Meer, 1993; van der Meer et al., 1995, 2012; van der Meer and Bowmaker, 1995; Maan et al., 2006) and molecular biological studies (Terai et al., 2002a, 2006; Carleton et al., 2005; Seehausen et al., 2008; Hofmann et al., 2009; Miyagi et al., 2012). Over the last decade, photoreceptors called visual pigments have been studied in terms of allele frequency in natural populations and in terms of their light-absorbing functions. In this section, we describe recent molecular studies on cichlid visual pigments.

The primary determinant of visual perception is the absorption of photons by visual pigments in retinal photoreceptor cells. In vertebrates, visual pigments consist of a light-absorbing component (the chromophore) and a protein moiety (the opsin) (Dratz et al., 1985; Shichida and Imai, 1998). Freshwater fish, including Lake Victoria cichlids, use either 11-*cis*-retinal (A1 retinal) or 11-*cis*-3,4-dehydroretinal (A2 retinal) as the chromophore (Yokoyama, 2000; Carleton et al., 2005). Absorption spectra of visual pigments depend on the interactions between the chromophore and the opsin. Changes in the absorption spectrum depend on the properties of the chromophore (A1 retinal or A2 retinal) (Harosi, 1994; Parry and Bowmaker, 2000) and/or on amino acids interacting with the chromophore (Yokoyama, 2000; Takahashi and Ebrey, 2003; Hunt et al., 2007).

African cichlids have eight opsin genes: one is for scotopic vision (vision under low light conditions; Rhodopsin, *RH1*) (Sugawara et al., 2002), the others are for color vision (*SWS1*, *SWS2A*, *SWS2B*, *RH2A α* , *RH2A β* , *RH2B*, and *LWS*) (Parry et al., 2005; Spady et al., 2006; Hofmann et al., 2009). The color opsins are sensitive to ultraviolet–violet light (short-wavelength sensitive, *SWS1*), blue light (short-wavelength sensitive, *SWS2A* and *SWS2B*), medium light (Rhodopsin like, *RH2A α* and *RH2A β*), and medium to red light (long-wavelength sensitive, *LWS*). Lake Victoria cichlids mainly express *SWS2A*, *RH2A* (putative *RH2A α* and/or *RH2A β*), and *LWS* (Hofmann et al., 2009) for color vision. Therefore, their perception of color signals depends on the properties of these three visual pigments: *SWS2A*, *RH2A* and *LWS*.

It was thought that Lake Victoria cichlids adapted their vision to the various ambient light environments (Fryer and Iles, 1972; Seehausen, 1996). Light environments in Lake Victoria are affected by water transparency and depth (Seehausen et al., 1997a, 2008). Dissolved substances such as mud particles, and tiny organisms generate turbid water conditions (low transparency), in which scattered short-wavelength light creates reddish light environments (with abundant long-wavelength light). In rocky shores, long-wavelength light is more abundant in deep water than in shallow water. Cichlid vision is important not only for mate choice (Fryer and Iles, 1972; Seehausen et al., 1997a, 2008; Seehausen and van Alphen, 1998) but also for food acquisition (Hori et al., 1993; Kohda and Hori, 1993; Kawanabe et al., 1997). Recently, the visual adaptation of Lake Victoria cichlids has been confirmed by molecular analysis of opsins as explained in the next section (Terai et al., 2006; Seehausen et al., 2008; Miyagi et al., 2012, see below).

Divergences of *LWS* alleles and nuptial colorations lead to speciation of cichlid species in rocky shores

The first molecular study of opsins in Lake Victoria cichlids was published in 2002 (Terai et al., 2002a). The authors analyzed sequences of opsin genes from 17 species and found that the amino acid sequences of the *LWS* gene were diversified among species, and that the alleles were fixed within species or populations (Terai et al., 2002a). According to these results, *LWS* was considered to be a candidate gene involved in visual adaptation and speciation in cichlids because most of the polymorphic sites were shared among species due to their low genetic diversity (Nagl et al., 1998, 2000; Terai et al., 2004). The adaptation of *LWS* pigments to light environments and the matching of color signals generated by nuptial colorations were analyzed further (Terai et al., 2006; Seehausen et al., 2008; Miyagi et al., 2012). Here, we review these studies and show that the diversification of coloration was caused by mate choice through adaptation of visual perceptions.

In the rocky shore-dwelling species *N. greenwoodi*, the frequencies of *LWS* alleles are differentiated between populations in habitats with high water transparency (dominated by the H allele) and those in habitats with low water transparency (dominated by the L allele) (Terai et al., 2006). The absorption spectrum of *LWS* pigments that was experimentally reconstituted from the *LWS* protein of the L allele sequence and A2-retinal was shifted 7 nm towards red from that of the H allele (Fig. 2). Since long-wavelength light is more abundant in low-transparency water than in high-transparency water, color vision with red-shifted *LWS* pigments can absorb ambient light more effectively in low-transparency water. Therefore, the *LWS* alleles of *N. greenwoodi* are adapted to ambient light environments. The adaptive *LWS* alleles have

LWS allele	Amino acid position							λ_{\max} (nm)	
	62	137	177	216	230	272	275	A1	A2
P	V	A	A	F	T	V	I	544 ^a	-
Sp	I							549 ^b	598 ^b
Py		G				M		551 ^b	598 ^b
M3				Y				553 ^b	601 ^b
H				Y	A		C	559 ^c	604 ^c
L		S	Y	A				558 ^c	611 ^c

Fig. 2. Maximum absorption spectra of visual pigments. Maximum absorption spectra (λ_{\max}) of (A) LWS pigments reconstructed from seven LWS alleles found in Lake Victoria cichlids. (A) The amino acid positions representing differences in amino acid residues among seven LWS alleles are shown, according to the sequences of LWS opsin. Amino acid replacements compared with the LWS-P allele are indicated by capital letters. The λ_{\max} of A1 retinal- and A2-retinal derived LWS pigments were measured by ^aSeehausen et al. (2008), ^bMiyagi et al. (2012), and ^cTerai et al. (2006).

diverged between populations by divergent selection, as shown by the analysis of selection pressure acting on the LWS gene (Terai et al., 2006).

Male nuptial colors are also differentiated between populations from habitats with high water transparency (Fig. 1C, blue-black morph) and those from habitats with low water transparency (Fig. 1D, yellow-red morph). In low-transparency water, the yellow-red morph effectively reflects light that is absorbed by the red-shifted LWS pigments in yellow to red light environments. Thus, the yellow-red morph is conspicuous in low-transparency water, and nuptial coloration may be differentiated by mate choice.

Hence, the colors of fish in different populations were shaped by sexual selection through local adaptation of visual perception. The differentiation of visual perception and nuptial coloration implies the divergence of color signals. The process of adaptive divergence of color signals caused by ecological selection is known as “sensory drive” (Endler, 1992; Boughman, 2002; Kawata et al., 2007). The divergence of color signals observed in *N. greenwoodi* is well matched with the process of sensory drive, and populations of *N. greenwoodi* may be at the incipient stage of speciation by sensory drive (Fig. 3A) (Terai et al., 2006).

A correlation between the adaptive divergence of LWS and divergence in male nuptial coloration has also been observed in *Pundamilia* species (Seehausen et al., 2008). *P. pundamilia* inhabits shallow water rocky shores, where blue light is abundant; and *P. nyererei* inhabits deep water rocky shores, where red light is abundant. The frequencies of LWS alleles in *P. pundamilia* (dominated by the P allele) and *P. nyererei* (dominated by the H allele) are differentiated. The absorption spectrum of LWS pigments reconstituted from the sequence of the P

allele is shifted 15 nm toward blue compared to that reconstituted from the H allele (Fig. 2). The blue-shifted P allele and the red-shifted H allele are adapted to light environments abundant in blue and red light components, respectively. Nuptial coloration is also differentiated between *P. pundamilia* (Fig. 1A, blue-gray) and *P. nyererei* (Fig. 1B, red), and these colors are conspicuous in blue and red light, respectively. Also in this case, the different colorations of species were shaped by sexual selection through local adaptation of visual perception, implying the divergence of color signals. The divergence of color signals observed in *Pundamilia* species is well matched with the process of speciation by sensory drive (Fig. 3B) (Seehausen et al., 2008).

Diversification of nuptial coloration may maintain species diversity in Lake Victoria

As mentioned, speciation is the process of establishment of reproductive isolation between species, and Lake Victoria cichlid species can potentially interbreed. Therefore, reproductive isolation among Lake Victoria cichlids, especially among coexisting species, is maintained by certain mechanisms of pre-zygotic isolation. The divergence of color signals among species is likely to provide a mechanism for pre-zygotic isolation, and Miyagi et al. (2012) investigated this possibility by using six coexisting species from broadly sandy/muddy bottom habitats without defining physical structures (e.g. rocks and vegetation). They analyzed eight opsin genes and found that LWS is the most variable, and that the frequency of one LWS allele is dominated in one species. Such dominated alleles were found in each of the species. Reconstitution of the LWS pigments showed that functional differences exist among species (Fig. 2A; H, M3, Py and Sp allele). Nuptial coloration also differs between species (Fig. 1, E-H) and is correlated with the peak absorption (λ_{\max}) of the LWS pigments in the six species (two species were not shown in Fig. 1). The divergence of visual perception and nuptial coloration indicates the divergence of color signals. Hence, the diversification of nuptial coloration may play a role in maintaining reproductive isolation among coexisting species through signal divergence (Fig. 3C). In Lake Victoria, cichlid species coexist in most habitats, possibly by overlapping distributions of two species with adaptation of their vision to different light environments or possibly by the visual adaptations to other ecological factors (e.g. types of the food). If color signal divergence plays a common role in maintaining pre-zygotic isolation, diversification of nuptial coloration maintains the species diversity of cichlid fish in this lake.

Conclusion and future prospects In this review, we demonstrated the importance of color in the adaptive radiation of Lake Victoria cichlid species. Fish coloration is produced by four types of chromatophores: mel-

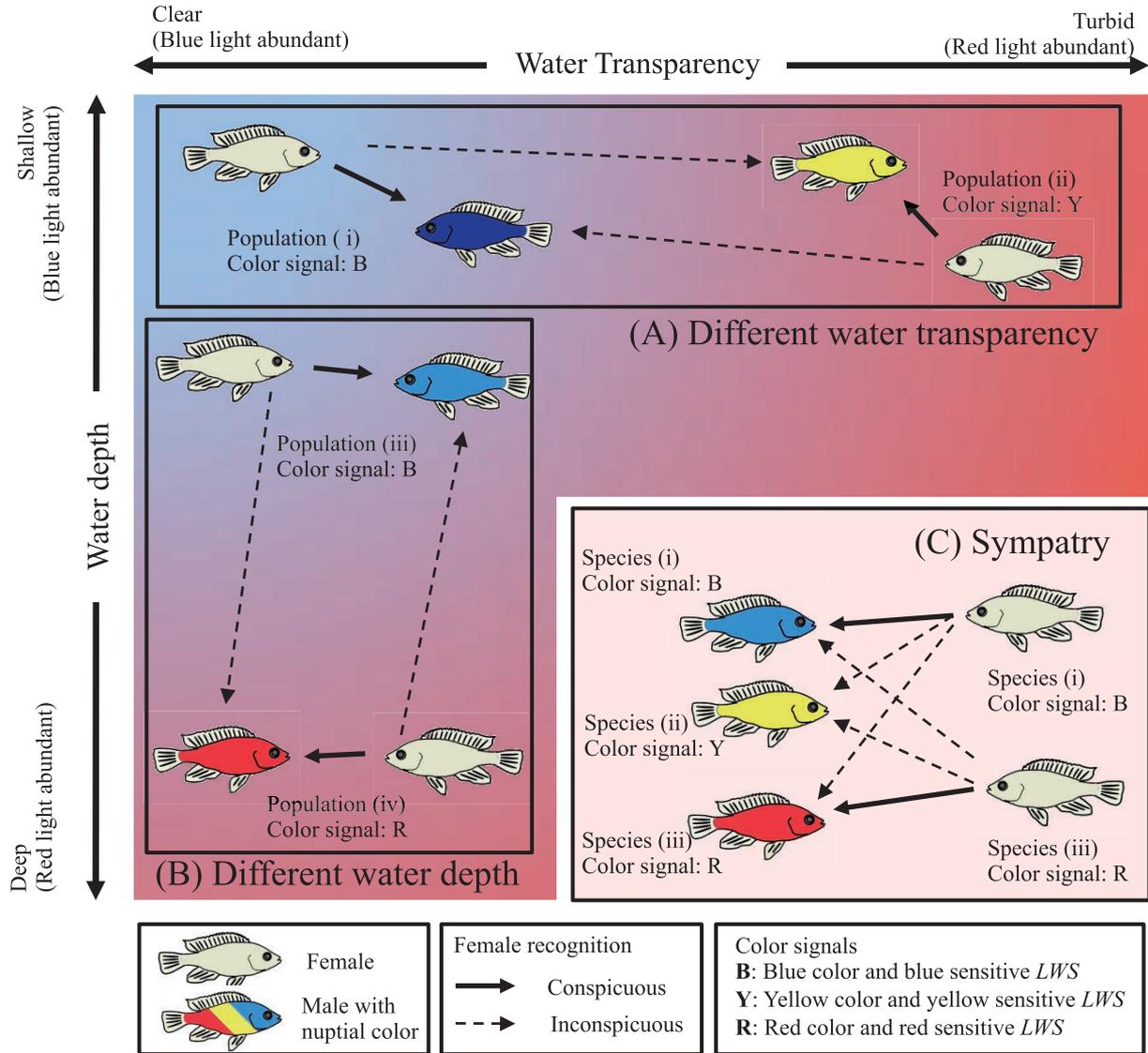


Fig. 3. Divergence of mating color signals among cichlid species in Lake Victoria. Divergence of mating color signals among cichlid species in Lake Victoria is shown in schematic figures. Female mating preferences via species-specific mating color signals, which are consistent with male nuptial color and sensitivity of LWS pigments, differ between species (or populations) inhabiting (A) different water depths, (B) different water transparencies, and (C) the same habitats (in sympatry). (A) and (B) LWS alleles are diversified among populations inhabiting different light environments and are adapted to the light environments in order to absorb ambient light efficiently. Male nuptial colors are also diversified among these populations to reflect ambient light efficiently, and males have evolved conspicuous colors to suit the adapted color vision of females. To match color signals, females select males with conspicuous colors (conspecific males) as mating partners, which generates reproductive isolation between populations and leads to speciation by sensory drive. (A) Females of population (i) (shorter wavelength-sensitive LWS) see males with the blue nuptial coloration of population (i) conspicuously and select these conspecific males as mating partners. By contrast, females of population (ii) (longer wavelength-sensitive LWS) see yellow males of population (ii) conspicuously and select conspecific males as mating partners. (B) Females of population (iii) (shorter wavelength-sensitive LWS) see males with the blue nuptial coloration of population (iii) conspicuously and select these conspecific males as mating partners. By contrast, females of population (iv) (longer wavelength-sensitive LWS) see red males of population (iv) conspicuously and select conspecific males as mating partners. (C) In sympatric habitats, mating color signals (combinations of male nuptial color and LWS) of coexisting species are diversified among species, and females select conspicuous males with matching color signals as mating partners, leading to the maintenance of species diversity in sympatry.

anophores, xanthophores, erythrophores, and iridophores. Mature male cichlids display their colors in the breeding season, and the main role of nuptial coloration is to attract females choosing mates. Coloration of the skin

reflects light and changes light components depending on the color, and the reflected light becomes a signal to attract females. Females receive the color signal via their visual system and find signals that are easier to

detect more attractive. The cichlid visual system is not used only to detect color signals, but also for food acquisition and to detect and avoid predators; therefore, the visual system is adapted to ambient light environments. Thus, male nuptial coloration has shaped and evolved to create signals more easily detectable by visual perception in females. Divergence of color signals by adaptation of visual perception to different light environments causes reproductive isolation between populations or species (Fig. 3, A and B). Furthermore, differences in color signals among coexisting species maintain reproductive isolation by preventing hybridization (Fig. 3C). In conclusion, the adaptation of visual perception to heterogeneous environments has generated diversity of nuptial coloration, and diversity of nuptial coloration has caused speciation and has maintained species diversity in Lake Victoria cichlids.

Understanding the genetic mechanisms underlying cichlid nuptial color formation is key to developing the study of species diversity in cichlids, but knowledge of which genes are involved in the color formation is still lacking. Recently, the genome of a Lake Victoria cichlid species was determined by the cichlid genome consortium. The genome information will be useful for the isolation of candidate genes controlling differences in male nuptial coloration between species. Genome-wide assays, such as RNA-seq analysis of colored skin by using the next generation of sequencers, could be applied to isolate candidate genes. These techniques could provide new insight into molecular mechanisms of the synthesis of cichlid pigment molecules, the formation of male nuptial coloration (distribution of chromatophores), and how sexual selection acts on the color formation genes that control differences in male coloration between species.

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