Standard Review

Mechanisms and molecular genetic bases of rapid speciation in African cichlids

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African cichlid fishes are a textbook model of evolution in motion but the molecular genetic bases and mechanisms involved in their rapid speciation largely remain elusive. Emerging experimental evidence now suggests that African cichlids have undergone rapid speciation due to a combination of their molecular genetic potential and the influences of the environment on this potential. The genetic potential of the cichlids lies mainly in the ecomorphological plasticity of their feeding apparatus and their strong sexual selection. Putative genes that underlie the phenotypic variations in African cichlids are beginning to be unravelled but their coverage in the literature remains modest and scattered. This review forms one of the first comprehensive attempts to consolidate emerging data that explain various genes and mechanisms underlying explosive speciation in this family of fishes. The review analyzes the modes of African cichlid speciation, radiation-in-stages model, molecular genetic bases of plastic pharyngeal jaws and teeth and signature genes for sexual selection premised mainly on nuptial colour patterns, egg dummies and maternal mouth brooding, opsins and the sensory drive hypothesis. Explaining sexual selection mechanisms based on colour patterns and sensitivity to light is crucial to understanding African cichlid species biodiversity and conservation in polluted lakes.

Key words: African cichlids, rapid speciation, sexual selection, genes.

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INTRODUCTION

In the last 10 million years, African cichlids (Family Cichlidae; Order Perciformes) have undergone such unexpected rates of speciation that they are now a model species for show-casing evolution in motion. Cichlid fishes are tropical freshwater fish that are unsurpassed by any other vertebrate group in terms of the sheer number

of species (> 3,000), variety of body shapes, assortment of colouration, behavioural diversity and degree of trophic and ecological specialisation (Fryer and Iles, 1972; Meyer, 1993; Stiassny and Meyer, 1999; Kornfield and Smith, 2000; Kocher, 2004).

Since the publication of the first reports on the East African fish fauna at the end of the 19th century (Boulenger, 1898a; 1898b), the exceptional diversity of fishes in the family Cichlidae has attracted the attention of evolutionary biologists. Cichlids were considered an aberrantly species-rich group, yet the variation endowed within the cichlids has made them a textbook model for the study of rapid speciation and diversification, and animal behaviour. The multiple invasion model suggests that each of the East African Great Lakes was colonized by multiple lineages which had evolved independently in time (Fryer, 1977) and space (Mayr, 1942). However, the model cannot adequately explain the explosive speciation and enormous diversity of African cichlids (Danley and Kocher, 2001).

Molecular analyses have revealed that the phylogenetic relationships among the major lineages of cichlids are consistent with an initially Gondwanaland distribution, with the Indian and Madagascar representatives forming the most basal lineages and the reciprocally mono-phyletic African and American lineages being sister groups. This distributional pattern is congruent with models of vicariance biogeography rather than overseas dispersal (Zardoya et al., 1996; Streelman et al., 1998; Farias et al., 2000, 2001; Sparks, 2004). Only about a dozen extant species represent the most basal para-phyletic lineages of cichlids from India/Sri Lanka and Madagascar, which are the two landmasses that split off first from the supercontinent of Gondwanaland between 165 and 130 million years ago (MYA). The Americas are inhabited by an estimated 400 - 500 cichlid species.

In Africa, the cichlid centre of biodiversity is in East Africa where they inhabit several large and small lakes and have formed the so-called "species flocks" with sometimes hundreds of endemic species in each of these lakes. The greatest diversity of cichlids is in Lakes Victoria, Malawi and Tanganyika where a total of > 2000 species occur (Salzburger and Meyer, 2004). With an age of 9 - 12 million years, Lake Tanganyika is the oldest of East Africa's Great Lakes (Cohen et al., 1993, 1997) followed by Lake Malawi with a probable age of 2 - 5 million years (Johnston and Ng'ang'a, 1990; Delvaux, 1995). Lake Victoria is the youngest, with an estimated age between 250,000 and 750,000 years (Johnston et al., 1996). These Lakes contain varying numbers of cichlid species: Lake Tanganyika (200-250 species), Lake Malawi (500 - 700, could even be 1000 species) and Lake

Victoria (500 or more) (Seehausen, 1996; Seehausen et al., 2003; Verheyen et al., 2003; Snoeks et al., 1994).

Although Lakes Victoria and Malawi have very high species richness and morphological diversity of cichlids and have been studied as model systems for explosive speciation and ecomorphological diversification, the rivers associated with Lake Victoria and Lake Malawi have low cichlid species richness and morphological diversity. This difference has been attributed to the observation that rivers lack the wealth of ecological opportunity that drives cichlid adaptive radiation in lakes (Joyce et al., 2005). In their study, Joyce et al. (2005) showed that the situation is different in Southern Africa. Haplochromine cichlids in 5 Southern African rivers (Upper Congo, middle/upper Zambezi, Okavango, Cunene and Limpopo) show species richness and ecomorphological diversity similar to that in Lake Victoria and Lake Malawi. Joyce et al. (2005) found numerous sympatric haplochromines different in shape and size within each of the rivers surveyed. Within this Southern African radiation, mitochondrial DNA haplotypes com-prise 6 clades within which a large number of closely related haplotypes have arisen from a small number of more divergent haplotypes.

On the other hand, several morphologically different and geographically distant (allopatric) species have very similar haplotypes. These phylogenetic relationships suggest a species flock that emerged rapidly and simul-taneously in many geographically distant rivers, in which very similar haplotypes are found several thousand kilometres apart, yet for which strongly differentiated haplotypes are found within single populations. Through the use of geological evidence, Joyce et al. (2005) contend that the high diversity among Southern African riverine cichlids arose from Lake palaeo-Makgadikgadi, a lake that disappeared about 2000 years Before Present. The centre of this extinct lake is now a saltpan north of the Kalahari Desert. They showed that Lake palaeo-Makgadikgadi hosted a rapidly evolving cichlid species radiation comparable in morphological diversity to that in the extant African Great lakes. This Lake palaeo-Makgadikgadi stock of cichlids later seeded all major river systems of Southern Africa with ecologically diverse cichlids.

Modes of African cichlid speciation

Although Charles Darwin proposed the mechanism of speciation in his book On the origin of species by means of natural selection (Darwin, 1859), the mechanisms through which new species are generated remain a major problem and the evolutionary forces and genes that drive speciation are still not well understood (Kocher, 2004). In the case of the African cichlids, biologists worldwide are still intrigued by their explosive speciation in the Great Lakes of East Africa. Mayr (1963, 1984) promoted allopatic

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speciation, a gradual divergence of populations with completely separate geographic ranges.

Evidence against allopatric speciation begun to mount when Meyer et al. (1990) established through mitochondrial DNA evidence that the enormous haplochromine cichlid species in Lake Victoria evolved from a single ancestral stock. Indeed after the publication of geological data that Lake Victoria was completely dry during the recent Ice Age about 12, 400 years before present (Johnston et al., 1996), it became even more untenable that over 500 species of cichlids could have evolved within Lake Victoria in such an extremely short evolutionary time frame. Thus the old hypothesis of allopatric speciation in Lake Victorian cichlids (Greenwood, 1964) was discarded because it could not account for the explosive speciation of cichlids in the lake (Seehausen, 1996).

It has become apparent that the great diversity of East African Great lakes cichlid species (the cichlid problem) contradicts traditional models of speciation (Salzburger and Meyer, 2004). There is now a wealth of evidence that parapatric (divergence of populations with adjacent geographic ranges) and sympatric speciation (divergence of populations in the same geographic area) also take place (Kocher, 2004). Despite abandoning the hypothesis of allopatric speciation, the question of how such large numbers of cichlid species (and not the other families of fishes found in these lakes) evolved rapidly into a large number of closely related but morphologically diverse species in such a short period of time continues to bother biologists. Hence recent studies in evolution and ecology have focused on selective forces that are responsible for the differentiation of populations regardless of the gene flow among incipient species (Kocher, 2004).

It is now becoming clear that sexual selection is an important force in the origination of new species (Higashi et al., 1999). Disruptive sexual selection on colour polymorphisms has caused sympatric speciation and accounts for the rapid evolution of cichlid diversity in Lake Victoria (Seehausen and Van Alphen, 1999). Intrinsic properties that cause disruptive selection elevate sympatric speciation rates (Seehausen and Van Alphen, 1999). But rapid radiations only occur in parts of the lake with relatively good visual conditions (Seehausen et al., 1997). When colour vision is impaired by water pollution and eutrophication, disruptive sexual selection becomes an ineffective driver of sympatric speciation (Seehausen and Van Alphen, 1999).

Male-male competition is also an important agent of diversification in African cichlids (Seehausen and Schluter, 2004). Closely related species of cichlids with very different colours are often sympatric or parapatric (Seehausen and Van Alphen, 1999; Danley et al., 2000). Competition between males for breeding territories promotes colour diversification thereby setting the stage for speciation (Seehausen and Schluter, 2004). Colourful males dominate breeding territories that they defend very fiercely against other males. Thus males of the same colour compete more heavily than males of different colours. This leads to different colour distribution patterns and can explain the origin of colour diversity and colour morphs (Seehausen and Schluter, 2004). Rare male colour morphs that increase in populations under negative frequency-dependent selection can lead to stable colour polymorphisms or sympatric speciation. Salzburger et al. (2009) discussed the possibility of acoustic, olfactory and behavioural cues in mate recognition and choice, but the genetic bases of these traits are still anectodal.

Radiation-in-stages model

Ecological and behavioural factors have had the largest effect on the diversification of African cichlids. The wealth of ecomorphological and behavioural traits that have led to the rapid speciation and diversity of African cichlids has now become known as the radiation-in-stages model. This 3-stage radiation model (Streelman and Danley, 2003) explains the extraordinary species richness of cichlids. The primary stage consists of divergence into different habitats. In the secondary radiation, ecomorphological differentiations occur and the tertiary stage consists of sensory behavioural diversification.

The first radiation resulted in the divergence of the rock-dwelling species from the sand-dwelling species. In Lake Malawi, this split resulted into about 200 species of rock-dwelling and sand-dwelling cichlids. This mechanism of cichlids species formation is consistent with allopatric speciation (Jonsson and Jonsson, 2001). Adaptation to the rock and sand macro-habitats resulted in the divergence of many morphological and behavioural characteristics such as body shape, trophic morphology, habitat preference, colour patterning and reproductive behaviours (Danley and Kocher, 2001).

In the second radiation, ecomorphological selection of the feeding apparatus led rock-dwellers to an adaptive innovative switch from the mandibular oral jaw to the pharyngeal jaw. Cichlids therefore acquired 2 sets of jaws: true mandibular oral jaws which shape the normal mouth to suck scrape and bite off bits off food and internal pharyngeal jaws derived from the fifth gill arch, found in the throat and used to mash, macerate, slice or pierce the morsel before it is ingested (Salzburger and Meyer, 2004). The innovations in the cichlid pharyngeal jaw led to the utilization of novel prey and ultimately to the trophic diversification of these fishes (Hulsey, 2006). Pharyngeal jaw modification is therefore a key innovation that determines what prey fishes exploit, hence some cichlids are molariforms that specialize in crushing molluscs, while others are papilliforms that utilize prey requiring less force to process (Hulsey, 2006). The specialized organisation of the pharyngeal jaw apparatus is a shared derived characteristic (synapomorphy) among

all cichlids (Liem, 1973).

The jaws are exceedingly versatile and adaptable and can change in form even within the lifetime of a single individual (Meyer, 1993). The oral jaw can grow specialized teeth which allow the cichlids to gather different types of foods. The division of labour between the oral jaws (mandibles) and the pharyngeal jaws has made cichlids become very efficient feeders, allowing them to capture and process a very wide variety of food. This specialized anatomy of the jaws is 1 morphological explanation given for the rapid speciation of cichlids. With the additional pharyngeal jaw, the mandibular oral jaw was afforded the evolutionary opportunity to diversify into specialized mouthparts for collecting different foods. Oral jaw specializations enabled the cichlids to exploit new food resources thereby allowing trophic diversification (Bootsma et al., 1996). Cichlid genera diversified in response to competition for trophic resources.

Feeding habits, dietary preferences and trophic morphological differences now account for the 10 - 12 genera of Lake Malawi cichlids (Danley and Kocher, 2001). The oral jaw has allowed a variety of foraging strategies. As a result, cichlids exploit a range of trophic niches usually occupied by several families, if not orders of fishes (Greenwood, 1964). Thus progression from ecological diversification to the refinement of trophic oral jaw structures is the major event in the secondary radiation of cichlids (Seehausen, 1996; Sturmbauer, 1998). This is consistent with the Darwinian adaptation of beaks to different food and ecological habitats in finches on the Galapagos Islands (Grant, 1981).

The third radiation, which resulted into the diversification of extant species, is mainly based on sexual selection (Danley and Kocher, 2001). While morphological features that evolved during the primary and secondary radiations have remained conserved, sexual selection now accounts for the incredible species richness of African cichlids. After elaborate experimentation, it was shown that cichlid fish species of Lake Victoria are sexually isolated by mate choice (Seehausen and Van Alphen, 1998, 1999). Male secondary sexual characteristics and female mating preferences determine mating behaviour. Sexual selection on male nuptial colours was a central driving force in the diversification of haplochromine cichlids in Lake Victoria (Seehausen and Schluter, 2004). It has been shown that direct mate choice of females for differently coloured males maintains reproductive isolation and diversity among sympatric cichlids (Seehausen, 1996). Females have a strong preference for males of a particular colour when light conditions are sufficiently good.

Competition for mates and assortative mating drives the diversification process. Mate choice by the females also allows full sympatric speciation as in the models of Lande (1982), Turner and Burrows (1995), and Payne and Krakaeur (1997). The abundance of sympatrically occuring colour morphs as well as the common absence of mating barriers other than behavioural ones suggests that sympatric speciation has played an important role in the explosive speciation and adaptive radiation of cichlids. However, the increased turbidity of the water due to pollution has broken down reproductive barriers leading to hybridization with other species, and a decline in cichlid species in certain parts of Lake Victoria (Seehausen et al., 1997).

With the increasing use of molecular biology techniques, the interest has now shifted towards the identification of genetic variation and isolation of putative genes responsible for phenotypic differences within and between closely related species. Thus genetic markers are being used to characterize population structure and trace the phylogenetic relationships among species.

Genetic bases of jaw and teeth morphology

Fish species that exploit hard or attached prey and those that feed on highly mobile prey, have evolved prescribed mandibular morphologies that correspond to the mechanical requirements of the feeding apparatus. Fish that prey on hard food evolve short and stout jaws for efficient biting, and those that eat mobile prey have developed gracile jaws for suction feeding (Albertson et al., 2005). Within this ecological prism, cichlids have used the biting-suction feeding instruments as a key point of evolutionary departure. The first step in elucidating the genetic architecture of the cichlid oral jaw apparatus was reported by Albertson et al. (2003a). They showed that skeletal differences in the head and oral jaw apparatus were inherited together, suggesting a degree of pleiotropy in the genetic architecture of this character complex. Alleles implicated in the evolution of jaw morphology were differentially distributed in cichlids that were algal scrapers and suction feeders. Hybrids showed that 4 to 11 genes were involved in the evolution of different jaw morphologies. The genes affect the shape of skeletal elements, although some of them were known to influence several other traits (Albertson et al., 2003b). Terai et al. (2002) found that a chromosomal region marked by polymorphism of the bone morphogenetic protein 4 gene (bmp4) contributes to variations in the shape of the upper and lower jaw elements in an explosively speciated lineage of East African cichlids. The same gene would later be shown to affect jaw morphology in mice (Wilson and Tucker, 2004). Consistent with the role of bmp4 as a putative genetic signature for the divergence of jaw morphology, Terai et al. (2002) found a high rate of amino acid substitutions in the pro-domain of the bmp4 protein.

The bmp4 gene is an attractive candidate for the evolution of craniofacial diversity in vertebrates and its regulation underlies some aspects of cichlid evolution. Albertson et al. (2005) demonstrated that bmp4 has the potential to alter mandibular morphology in a way that mi-

mics adaptive variation among cichlid species. Increased levels of *bmp4* were associated with biting /crushing morphologies; hence *bmp4* is a major player in the evolutionary drive from gracile to robust jaws. The *bmp4* gene has also been found to be important in determining beak shape diversity among avian species, and interestingly, *bmp4* signalling is thought to have contributed to the evolution of beak shape in Darwin's finches (Albertson et al., 2005).

The range of dental diversity in Lake Malawi cichlids is amazingly high: some species possess about 10 teeth in a single row, or as many as 700 teeth in up to 20 rows (Fraser et al., 2008). Cichlid teeth are as diverse as their jaws and are essential components of the trophic machinery. Cichlid species also differ in tooth size, spacing and shape. In terms of tooth shape, some cichlids are unicuspid, bicuspid or tricuspid species. Tooth shape is highly correlated with the feeding ecology of cichlid fishes (Kocher, 2004). A unicuspid dentition is suited to generalist feeding; hence cichlids with unicuspid teeth are piscivorous, zooplanktivorous and insectivorous species, while those with tricuspid teeth are specialized algal scrapers (Fraser et al., 2008). Tooth development is a classic example of tissue and genic interactions. In vertebrates, there are genes that are essential for teeth development. For example, gene s*hh* is crucial for correct establishment of the global dental programme (Fraser et al., 2008). It is expressed during tooth morphogenesis and marks the bell-shaped dental epithelium. Fraser et al. (2008) showed that a combination of *shh* and *pitx2* were necessary for a competent field of tooth initiation.

Tooth shape differences between bicuspids and tricuspids are choreographed by a small number of genes. Cuspid number and morphology are regulated by antagonistic actions of extracellular signalling ligands such as fibroblast growth factors (fgfs) and bone morphogenetic proteins (bmps) secreted from transitory enamel knots (EKs) (Streelman et al., 2003a; Fraser et al., 2008). These proteins are active during tooth initiation and morphogenesis and affect the sites in the jaw where teeth develop, as well as the shape of individual teeth. During tooth initiation, the expression of genes *bmp4* and *fgf8* in the epithelium control the mesenchymal expression of genes *pax9* and *msx1*, which direct tooth formation and position. The expression of gene *bmp4* in the mesenchyme promotes formation of the primary EK. Then bpms and fgfs secreted from the EK modulate cusp development (Fraser et al., 2008); b*mp4* specifies unicuspid dentition and *fgf8* codes for multicuspid dentition (Streelman and Albertson, 2006). Both unicuspid and multicuspid teeth develop under the control of homeobox genes expressed in the mesenchyme; *msx1/2* for unicuspids and *barx1*, *dlx1/2* and *lhx6/7* for multicuspids.

It has been suggested that divergent dentitions in cichlids were driven by the genes *pitx2*, *eda* and *wnt7b* and and their interactions with *shh* and *edar* (Fraser et al.,

2008). The genes *eda* and *wnt7b* regulate initial tooth germ size and position within rows. These genes are responsible for putting tooth rows in jaws and teeth in tooth rows. Fraser et al. (2008) suggested that initiation of new tooth rows follows a copy and paste mechanism where the dental expression network is redeployed for each new tooth row. Later, it was demonstrated that a core gene network involving *bmp2*, *bmp4*, *dlx2*, *eda*, *dlx2*, *edar*, *pax9*, *pitx2*, *runx2*, *shh* and *wnt7b* is expressed commonly on cichlid oral and pharyngeal jaws (Fraser et al., 2009). These genes are core markers of dental epithelial initiation and are associated with variations in oral jaw tooth row number, tooth number within rows and the spacing of teeth. It was postulated that this core dental network represents a conserved set of molecules for tooth development, adding that it was likely that nature had never made a tooth without this core genetic network (Fraser et al., 2009).

Signature genes for sexual selection

Sexual selection is a special case of natural selection. Sexual selection acts on an organism's ability to successfully copulate with a mate. Selection makes many organisms go to extreme lengths for sex, often evolving elaborate body features to lure mates. In Lakes Malawi and Victoria cichlids, females select their mates based on male nuptial colouration (Seehausen and Van Alphen, 1998). Thus there is a possibility that female choice of male nuptial colours is a special driving force for speciation (Seehausen et al., 1999). It has been postulated that female cichlids have preferences for different male colours and that these female preferences cause reproductive isolation between incipient species.

Several genes carry the signatures of sexual selection and speciation in African cichlids. Signature genes that underlie phenotypic differences in cichlids can be located using genetic mapping techniques. It is now possible to locate chromosomal regions responsible for any quantitative trait in cichlids. Putative genes that confer phenotypic differences among various species are identified by positional cloning. Here, we review the putative genes that elucidate the molecular genetic raw materials that fuel phenotypic variations, explosive speciation and evolutionary success of African cichlids.

Colour patterns

Closely related species of cichlids exhibit different colour patterns that in turn direct the choice of mating partners (Seehausen and Van Alphen, 1998). The orange-blotch (OB) pattern is found in many species of Lakes Victoria and Malawi cichlids. After conducting many genetic crosses, Seehausen et al. (1999) proposed that OB is produced by an X-linked gene that is modified by an autoso-

mal locus in Lakes Victoria and Malawi cichlids. A region of conserved synteny has been found around the *OB* locus with a strong association to the *cski* marker located 2 cM from *OB*. Streelman et al. (2003b) also found that the *OB* locus was tightly linked to the *c-ski1* gene. Terai et al. (2002b) found a high rate of splicing events in the *hagoromo* gene, a putative gene involved in the development of pigment patterns in *zebra fish*. This gene has a complex pattern of alternative splicing which produces numerous splice variants in each species (Terai et al., 2003). These splicing patterns seem to be species-specific but the differences in splicing are yet to be linked to specific colour patterns.

Egg-dummy and maternal mouth brooding genes

One characteristic feature of haplochromines is their possession of egg-spots on the anal fins of males (Goldschmidt, 1991). These egg-spots mimic real eggs and are therefore called egg-dummies. The egg-dummies play an important role in the mating behaviour of these maternal mouthbrooding fish (Hert, 1989) and are now known to have mediated the speciation of African cichlids (Goldschmidt and de Visser, 1990). Haplochromine egg-dummies form in juvenile male fish and begin to brighten when the young males reach sexual maturity. Anal fin egg-dummies also form in females but they are less colourful than those in males. In most riverine and rock-dwelling haplochromines, the egg-dummies are made up of a conspicuous yellow-red central area and a less trans-parent outer ring.

A female with mature eggs approaches the territory of courting males and lays eggs on the lake bed. Later the female swallows the eggs in their mouth. The female then pursues a male with bright anal fin egg-spots or egg dummies. The female tries to ingest the egg dummies and in the process she brings her mouth closer to the genital opening of the male. The male then releases sperms into the female's mouth thereby fertilizing the eggs. The molecular genetic basis of this elaborate inheritable mating behaviour has now been traced to the formation of pigment cells.

The egg-spots consist of pigment cells called xantho-phores (Salzburger et al., 2007). Using fluore-scent-based detection methods, reverse transcriptase PCR and *in situ* hybridization experiments, it has recently been shown that colony-stimulating factor I receptor a (*CsfI ra*) is the gene that mediates the production of yellow xanthophores in male haplochromine egg-dummies (Salzburger et al., 2007). *CsfI ra* is a type III receptor tyrosine kinase gene which is 2.9 kb long and has 21 exons. It has a cysteine-rich extracellular ligand-binding domain composed of five immunoglobulin-like chains, a transmembrane domain and an intracellular domain with two separate tyrosine kinase domains (Salz-burger et al., 2007).

Receptor tyrosine kinases are key components of cell

signalling networks and play crucial roles in physiological processes. These signal transduction cascades detect, amplify, filter and process a variety of environmental and intercellular cues. Their N-terminal extracellular domain binds ligands such as growth factors and hormones. Their C-terminal domain has kinase activity whereby transfer of a phosphate to tyrosine residues activates a signal trans-duction cascade that leads to xanthophore pigment gene expression.

2 important molecular genetic architectures and mechanisms could have accelerated the evolution of egg-dummies in haplochromines:

(a) Adaptive sequence evolution: The *CsfI ra* gene has more non-synonymous substitutions than synonymous ones (Salzburger et al., 2007). By definition, non-synonymous mutations easily change the translated amino acid sequence of proteins because they are under constant evolutionary pressure (Schattner and Diekhans, 2006). Sequence comparisons of haplochromine and non-haplochromine species revealed that several regions of the *CsfI ra* gene give a dN/dS ratio of greater than one, demonstrating that positive selection pressure has greatly changed the *CsfI ra* protein in haplochromines (Salzburger et al., 2007). The many variant forms of the *CsfI ra* protein suggest that novel modifications of existing signal transduction mechanisms evolved in haplochromines. This provided the genetic raw material which produced many routes in the expression of xanthophore pigment genes and resulted in an enormous variety of egg-dummies that characterise the explosive speciation of haplochromines.

(b) Duplication of receptor tyrosine kinase paralogons: Gene and genome duplications are important mecha-nisms for the evolution of phenotypic complexity, diver-sity, innovation, and origin of novel functions in the development of organisms (Ding et al., 2008). In many teleosts, several genes (*mitf*, *sox10*, *tyrosinase*) involved in pigment cell development are retained in duplicate copies (Hoegg et al., 2004) and this explains why teleosts possess greater diversity in colouration than tetrapods (Bagnara, 1998). In the cichlids of the East African Great Lakes, the *csfI ra* gene has also undergone several duplications (Braasch et al., 2006). It is now accepted that genome duplications and the expansion of the receptor tyrosine kinase signalling cascade proteins increased the repertoire of pigment cells that led to the enormous diversity pigment cell innovations found in cichlid egg-dummy patterns. The *pdgfr β-csfI r* locus was also thought to mediate new gene functions that could further drive the evolution of cichlid colouration (Braasch et al., 2006).

Opsin genes

Differences in opsin visual pigments alter visual sensitivity and dictate mate preferences. In Lake Malawi cichlids, an

ultraviolet (UV)-sensitive cone pigment was found to detect the UV reflectance common to many blue cichlids (Carleton et al., 2000). Terai et al. (2002) and Sugawara et al. (2002) found the signatures of positive selection for rhodopsin and long wavelength sensitive opsin genes. These genes allowed cichlid visual pigments to evolve rapidly and adapt to changing water quality parameters in different habitats of the lake. Consistent with this theory, Seehausen et al. (1997) showed that differences in visual sensitivity have influenced the evolution of colour patterns and sexual selection in cichlids adapted to living in turbid and clear parts of Lake Victoria.

Sensory drive hypothesis

Sensory drive is a hypothesis about how communication signals are designed to work effectively (Boughman, 2002). In the case of mating signals, the hypothesis explains how signals are best designed to attract mates. Thus the sensory drive hypothesis predicts that females mate more often with male phenotypes that they detect more easily and evolve to prefer signals that are conspicuous and easy to detect in the environment (Kawata et al., 2007). In guppies, orange spots are a visual cue in female mate choice (Endler, 1991; 1992). In sticklebacks, females prefer males with a larger area of red (Boughman, 2001; 2002). Both of these visual mate choice scenarios are influenced by environmental light (Gamble et al., 2003).

According to the sensory drive hypothesis, easy-to-detect signals are likely favoured by the choosy mates (Boughman, 2002). Thus females (usually the choosy ones) often prefer signals that are conspicuous, for example, long feathers, bright colours, complex vocalizations, or bizarre extensions of male morphology such as horns and eye stalks (Boughman, 2002). Inherent properties of signals such as their colour, intensity, or size, affect signal conspicuousness and detection by females (Boughman, 2002), including the following 3 processes described by Endler (1993):

(1) Habitat transmission (passage of signals through the habitat).
(2) Perceptual tuning (perceptual adaptation to local habitat).
(3) Signal matching (matching of male signals to female perception).

The sensory drive hypothesis explains how these three processes shape the evolution of inherent signal properties.

In cichlids, Gray and Mckinnon (2007) and Chunco et al. (2007) postulated that sensory drive could cause the appearance of colour polymorphisms and eventually lead to speciation (Kawata et al., 2007) even when the population is not geographically isolated (Seehausen et al.,

2008). Such evolution of sympatric species was earlier shown to occur in the sticklebacks of British Columbia (Boughman, 2001). According to Kawata et al. (2007), sensory drive hypothesis argues that divergent sensory adaptation in different habitats may lead to pre-mating isolation upon secondary contact of populations.

Speciation by sensory drive has traditionally been treated as a special case of speciation as a by-product of adaptation to divergent environments in geographically isolated populations (Kawata et al., 2007). However, if habitats are heterogeneous, local adaptation in the sensory systems may cause the emergence of reproductively isolated species from a single unstructured population. In Lake Victoria, habitat heterogeneity has been attributed to differences in ambient light regimes caused by water pollution and eutrophication (Seehausen et al., 1997).

In Lake Victoria cichlids, females prefer males with colours which the females perceive as intense or conspicuous (Seehausen and Van Alphen, 1998). Colour perception is determined by several different components (Kelber et al., 2003), one such component is sensitivity at a given wavelength of light (Kawata et al., 2007). Retinas of cichlid fish inhabiting relatively blue-shifted environments are more sensitive to blue light than the retinas of those from red-shifted environments (Carleton et al., 2005). Closely related species of cichlids in Lake Victoria differ in their retinal absorption spectra (Meer and Bowmaker, 1995). Cichlids with different retinal absorption spectra also have different male mating and breeding colouration (Kawata et al., 2007). In fact, the major peaks in the retinal absorption spectra match the most common breeding colours (Kawata et al., 2007). These observations had led earlier workers to the suggestion that evolution of the visual system could drive the speciation process in cichlids (Seehausen et al., 1997). Recent evidence now shows that in single unstructured populations of cichlids, sensitivity to light of different wavelengths is determined by heritable variation in the absorption spectra of opsins (Carleton et al., 2005; Mann et al., 2006). This can be demonstrated via three mechanisms of colour tuning.

First of all, changing the amino acid sequence of opsin genes, the so-called spectra-tuning, causes changes in peak absorption spectra of the visual pigments (Yokoyama and Radlwimmer, 2001). Fixed genetic differences were found in the *LWS* opsin locus between closely related populations of Lake Victoria cichlid fish (Terai et al., 2002). Variation in the amino acid sequence of opsin proteins may lead to differences in mate choice signals (Terai et al., 2002).

Secondly, colour vision can be changed by varying the transcription and translation of different opsin genes. When a larger amount of LWS opsin protein is expressed in the retina than the medium and short wavelength-sensitive opsins, the individual could be more sensitive to light of longer wavelengths (Kawata et al., 2007). In Lake

Malawi cichlids, Carleton et al. (2008) also found that differential expression of unique subsets of cone opsin genes produces drastic differences in the visual pigments of these fishes. Cichlids have 5 - 6 cone opsins but express only 3 of them in adults (Kawata et al., 2007). Some species of Lake Malawi cichlids that live in different light environments express complimentary subsets of opsin genes (Carleton and Kocher, 2001).

Thirdly, Carleton et al. (2008) suggested that cichlids have 4 cone opsin genes but additional duplications have produced 7 distinct cone opsin genes (*sws1*, *sws2b*, *sws2a*, *RH2b*, *RH2aβ*, *RH2aα* and *LWS*) which produce visual pigments that are spectrally distinct from each other.

Accordingly, 3 molecular genetic mechanisms produce differences in the visual pigment genes and spectral tuning; amino acid substitutions, differential expression and gene duplications of cone opsins. Given these 3 mechanisms, evolutionary adaptation of the visual system could lead to the divergence between populations in female preference for male nuptial colour (Kawata et al., 2007). Upon secondary contact, such divergence can cause pre-mating isolation and sympatric speciation by sexual selection (Herder et al., 2006; Schliewen et al., 1994; Seehausen and Van Alphen, 1999; Shaw et al., 2000). This happens as follows (Kawata et al., 2007):

(1) Spectral sensitivity evolves as an adaptation to environmental (ambient) light regimes.
(2) A female prefers to mate with a male whose nuptial colour reflects at the wavelength that she most intensely perceives.
(3) The female's sensitivity to light of a given wavelength depends on the absorption spectra of her visual pigments.

Recently, Seehausen et al. (2008) have demonstrated the sensory drive hypothesis in island populations of cichlid fish. They determined ecological, population genetic and molecular bases of divergent evolution in the cichlid visual system and linked it to divergence in male colouration and female mate preferences and finally demonstrated that this plethora of ecological and molecular forces can cause differentiation of neutral loci and reproductive isolation in sympatric cichlids inhabiting different light gradients in Lake Victoria (Seehausen et al., 2008). Male fish living at different water depths develop different colours and females prefer conspicuously coloured males due to variations in the *Long-wavelength sensitive* (LWS) opsin gene. Females have mating preferences for conspicuously coloured males. The LWS opsin genotype determines female mating preferences. Divergence in *LWS* systems is associated with divergence in male colours and female mating preferences. This causes differentiation of neutral loci, leading to reproductive isolation and speciation.

A 4-step sensory drive speciation model has been pro-

posed by Seehausen et al (2008):

(1) Divergent natural selection between light regimes at different water depths acts on *LWS*.
(2) Sexual selection for conspicuous colouration also becomes divergent because perceptual biases differ between light regimes.
(3) Their interaction generates initial deviation from linkage equilibrium between *LWS* and nuptial colour alleles.
(4) Subsequent disruptive selection due to reduced fitness of genotypes with a mismatch between *LWS* and colour alleles causes speciation, possibly involving reinforcement-like selection for mating preferences where male nuptial colour serves as a marker trait for opsin genotype. See Box 1 for a simpler synthesis of the sensory drive hypothesis in African lake cichlids.

Box 1: A model on sensory drive in African lake cichlids

The Seehausen et al. (2008) model explains how the physics of light predisposes African lake cichlids to find mating partners and then over time, drives the cichlid population towards sexual selection and sympatric speciation. Water tends to absorb red light and leaves blue light to travel to the bottom. This explains why deep water bodies appear blue. But in a slightly polluted and cloudy lake, blue light dominates the visual environment near the surface (because the blue light is absorbed by debri), while red light dominates the visual environment in deeper waters.

Cichlid fish populations live along a slope, from shallow waters at the lake's shores to deeper waters at the bottom of the lake. Thus some cichlids spend more of their time in blue light near the surface and red light in deeper waters. These differences induce genetic variation, allowing cichlids in shallow water to have genes that enable them to perceive blue light and deeper-dwelling cichlids are imbued with genes that favour them to see red light. Thus shallow-dwelling cichlids have genes that confer blue-light advantage and deeper water cichlids have genes that confer red-light fitness.

Therefore in different parts of the cichlid habitats, specific colour-sensitive genes are favoured by natural selection. Over many generations and if the cichlids do not move a lot within their range, blue-sensitivity will be common in cichlids near the surface and red sensitivity will prevail in cichlids in deeper water further down the slope. This natural selection acting on light sensitivity can divide the cichlid population and together with sexual selection, the divergence is exacerbated even further. Since female cichlids choose brightly coloured males for mating, it follows therefore that blue males living in deep water have difficulties finding female mates, for two reasons; there is little blue light in deep bottom water, making blue male fish appear duller than the red males and females in

deep water are less sensitive to blue light.

For these 2 reasons, bottom-dwelling blue male cichlids are doomed to a useless sex life and so are the red male cichlids living near the surface. After many cycles of selective breeding, the cichlid population will diverge into 2 different sub-populations that prefer to mate with fish of similar colouration, light sensitivity and habitat. Female cichlids will tend to prefer males of their own habitat because their colours are congruent to their colour sensitivity. In the longer term, the 2 sub-populations of cichlids will no longer mate with each other and will evolve into separate species. (Adapted from: http://evolution.berkeley.edu/evolibrary/news/090310_cichlidsspeciation)

Concluding Remarks

Studies in African cichlids are just beginning to unravel the interactions among genotype, phenotype and the environment. Molecular genetic studies, in particular have focussed on determining the ecological and evolutionary mechanisms that drive the speciation of cichlids in African lakes and rivers. These studies have illustrated the species richness and ecomorphological diversity of cichlids and documented various selective pressures involved in the ecomorphological differentiation and species divergence of African cichlids. The rapid speciation of African lake cichlids is now firmly rooted in their sexual selection and choice of mating partners based on nuptial colour patterns. Putative genes that underlie phenotypic variation and speciation of African cichlids are being elucidated. This research presents the potential to discover the full range of the cichlids species in African lakes and rivers.

Many cichlids, particularly the tilapias, are important food fishes, while others are valued game fish for anglers. Many cichlid species are also highly valued in the aquarium trade (Loiselle, 1994; Chapman, 1992). Cichlids are also the family of vertebrates with the largest number of endangered species, most of these found in the haplochromine group. According to the 2007 International Union for Conservation of Nature and Natural Resources red list, 156 cichlid species are currently listed as vulnerable, 40 species are listed as endangered, while 69 species are listed as critically endangered. 6 species, *Haplochromis ishmaeli*, *Haplochromis lividus*, *Haplochromis perrieri*, *Paretroplus menarambo*, *Platytaeniodus degeni* and *Yssichromis* sp. *nov.* 'argens' are extinct in the wild, while at least 39 species, most from the genus *Haplochromis*, have become extinct since the early 1990s (IUCN, 2006).

In the past few decades, the number of Lake Victoria cichlid species has declined and many species have collapsed into each other. Deforestation, nutrient run-off into the lake, eutrophication and pollution has conspired to make the lake's water more and cloudier and murkier.

Poor water quality makes it very difficult for female fish to choose mates based on colour, making the cichlid species that rely on visual signals for mate choice to interbreed. Ultimately, interbreeding has resulted in fewer species of cichlids. The evidence presented here points to how cichlid species biodiversity can be diminished by poor water quality emanating from human pollution. Given that cichlids are commercially important food fishes in Africa, current studies that focus on molecular genetic bases and mechanisms of speciation in cichlids will aid in correct decision-making in fisheries management, conservation, breeding and aquaculture.

Further work is still needed to map genes for phenotypic traits associated with speciation, such as jaw morphology and adult colour patterns. The goal is to identify the genes responsible for speciation of these fishes, and to study the geographic distribution of allelic variants among populations and species in the wild. A key element to studying the diversification in cichlids is the development of genomic resources to support the identification of genes underlying the phenotypic differences among species. The idea to sequence the whole genome of an African cichlid would help decipher the full range of cichlid genes and help shed more light on the molecular genetic bases of speciation. The current work involving the sequencing of the Nile tilapia may help reveal genomic hotspots responsible for the cichlids' ability to adapt and to diversify (Salzburger, 2009). The 'cichlidomics' era is now at hand. Further work to elucidate the signal transduction pathways involved in the expression of speciation genes is also needed.

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