Plastic fish in troubled waters

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DOI:
10.33612/diss.245585035

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2022

[Link to publication in University of Groningen/UMCG research database](#)

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Chapter 1

General Introduction

Elodie Wilwert

Species divergence may emerge as a result of local adaptation to heterogeneous environments (i.e. ecological speciation). Sensory systems are often a key parameter in local adaptation. In this thesis, I aimed to contribute to our current understanding of the mechanisms of sensory adaptation. Specifically, I aimed to explore how organisms adapt to local environments and how individual flexibility may contribute to evolutionary change. The fish visual system provides an excellent opportunity for this, as it shows extensive variation among species and populations. In this thesis, I focussed on cichlid fish, one of the most species-rich vertebrate families. I explored variation in visual system properties generated at the level of the eye, either genetically based or by plasticity, in order to evaluate whether adaptations of the visual system drive or contribute to reproductive isolation in East African cichlids. In particular, I explored 1) the potential contribution of differential chromophore usage to visual adaptation; 2) the contribution of developmental and environmental plasticity to the different components of the visual system; 3) the correlation between visual system properties and individual behaviour and, finally, 4) the relationship between changes in visual niches and speciation rates.

Speciation – Speciation, generally defined as the formation of reproductively isolated populations from an originally interbreeding population (Mayr 1963; Schluter 2000), can be driven by evolution, the process that results in the accumulation of genetic changes over time. As reproductive isolation evolves, gene flow between the populations is reduced, resulting in genetic differences between populations over time (Coyne and Orr 2004; Futuyma 2009). Unravelling the mechanisms that generate and maintain the immense diversity of species has been challenging evolutionary biologists for decades (Coyne and Orr 2004). New species may emerge as a result of several mechanisms: non-selective (e.g. genetic drift, polyploidization, founder-events; reviewed in Coyne and Orr 2004) and/or selective forces: natural selection – *the struggle to survive* – (Darwin 1859) and sexual selection – *the struggle to reproduce* (Darwin 1871). Divergence may be driven by uniform-selection, where populations experience similar selection and evolve similar phenotypes by fixing different advantageous genetically-based adaptations, (Schluter 2001) or ecological speciation (Schliewen *et al.* 1994; Schluter 2000; Rundle and Nosil 2005).

Ecological speciation – Environmental differences can play a key role in the formation of barriers that prevent gene flow. Local adaptation to heterogeneous environments may lead to the evolution of reproductive isolation and ultimately the formation of ecologically divergent species (Schliewen *et al.* 1994; Coyne and Orr 2004). Any type of reproductive barrier, including premating barriers (e.g. mechanical isolation, behavioural isolation) or post-mating barriers (e.g. genetic incompatibility between species that result in the sterility or inviability of hybrid offspring) may result in ecological speciation. Ecological speciation can be rapid (i.e. a few thousand generations; Hendry *et al.* 2007) with growing support in young adaptive

radiations (i.e. evolution of phenotypic and ecological diversity within a rapidly multiplying lineage; Schluter 2000). Adaptive radiations include classical examples such as Darwin's finches on the Galapagos islands (Lack 1947), three-spined sticklebacks in young Lakes of British Columbia (Rundle *et al.* 2000; Taylor and McPhail 2000) or cichlid fish (which are the focus of this thesis; reviewed in Turner 2007).

Phenotypic plasticity – One factor that might influence the process of ecological speciation is phenotypic plasticity. Phenotypic plasticity is defined as the "ability of a single genotype to produce distinct phenotypes when exposed to different environmental conditions" (West-Eberhard 2003a; b; Pigliucci *et al.* 2006; Ghalambor *et al.* 2007). As such, phenotypic plasticity allows organisms to rapidly adjust to fluctuations in a broad range of biotic and abiotic factors including for instance temperature, nutrition, pressure, light, parasites or competitors (DeWitt and Scheiner 2004; Gilbert and Epel 2009; 2015). Phenotypic plasticity is universal ranging from bacteria to multicellular organisms and has extensive variation in the extent, nature and developmental timing across species (e.g. Chervinski 1983; Gotthard *et al.* 1999). It encompasses, amongst others, anatomical structures, physiological processes and behaviour. Examples include predator-induced structural defences in water fleas, the gaudy commodore butterfly, *Precis octavia*, that varies in colour depending on temperature (reviewed in Pfennig *et al*. 2010) or colour changes during contests in the chameleon (e.g. Ligon and McGraw 2013).

The role of phenotypic plasticity in evolution is disputed (Bradshaw 1965; Schlichting and Pigliucci 1998; Agrawal 2001; Pigliucci 2001; West-Eberhard 2003a; b; DeWitt and Scheiner 2004; Pfennig *et al.* 2010; Ghalambor *et al.* 2007). On the one hand, plasticity might shield organisms from natural selection and thus prevent adaptive evolution (Huey *et al.* 2003, Price *et al.* 2003, DeJong 2005; Nonaka *et al.* 2015). On the other hand, it might promote adaptive evolution by allowing a broad tolerance to environmental conditions and thereby facilitating the colonization of novel ecological niches through rapid adjustment of the phenotype to local conditions. Such local adaptations can enable natural selection to enhance existing differences between phenotypes, promoting assortative mating and leading to reproductive isolation (Schlichting 1986, West-Eberhard 1989, Scheiner 1993; Pfennig *et al.* 2010).

Sensory adaptation – Sensory systems play a critical role in survival and reproduction. An enormous diversity of sensory system properties can be found across taxa (Stevens 2013). For instance, honeybees are able to discriminate between many flower colours (Arnold and Chittka 2012), while cave-dwelling fish have lost their eyes and entirely rely on chemical (smell/taste) communication (Greenwood 2010). Local adaptation of sensory systems to different environments may cause pre-mating isolation on secondary contact of populations and the

origin of new species (Endler 1992; Boughman 2002). In addition to evolutionary changes, sensory capacities can adjust to short-term changes in the environment without genetic change (i.e. plasticity). For example, in rainbowfishes, exposure to altering water flows results in a shift in neuromast (i.e. cells responsible for water motion detection) (Kelley *et al.* 2017).

East-African cichlid fish – Cichlids have become a model system for investigating how organisms adapt to various environments, both through phenotypic plasticity and evolutionary change (e.g. Henning and Meyer 2014; Carleton 2020). Cichlid fish are widely distributed ranging from South and Central America to Cuba, Africa, Madagascar, Arabia and India (Kocher 2004, Seehausen 2015) and with more than 2000 described species they represent one of the most species-rich vertebrate families (Kocher 2004). Not only are cichlids known for their large diversity in colour, diet and breeding strategy, but also their diversity in visual system properties (Carleton and Kocher 2001; Seehausen *et al.* 2008; Terai *et al.* 2017; Carleton *et al.* 2020; Carleton and Yourick 2020). They inhabit water bodies ranging from swamps and rivers to lakes (i.e. crater lakes, rift lakes, shallow and deep lakes). Spectral conditions vary substantially within water bodies. For instancse, in shallow waters, light conditions include all wavelengths of the sunlight spectrum. The absorption of light spectrum depends on the amount of dissolved and suspended organic material: in deep waters, these effects accumulate resulting in spectrum changes:

In clear waters, the extremes of the spectrum are progressively absorbed (orange-red light) or scattered (ultraviolet), resulting in a blue-dominated light spectrum at greater depths. In turbid waters, due to high levels of dissolved and suspended organic material, light conditions shift towards longer wavelengths with increasing depth, i.e. green, yellow and red (Figure 1). Cichlid fish inhabit a wide range of visual niches: some cichlid species are specialised to narrow depth ranges (i.e. specialists), while others inhabit a broad depth range (i.e. generalists) (Seehausen *et al.* 1996; Konings 2001; Konings 2015). Evidence shows that such heterogeneous photic environments drive adaptive evolution of visual systems in cichlid fish (e.g. Kocher 2004). In this thesis, I focused on cichlid species from Lake Tanganyika and Lake Victoria.
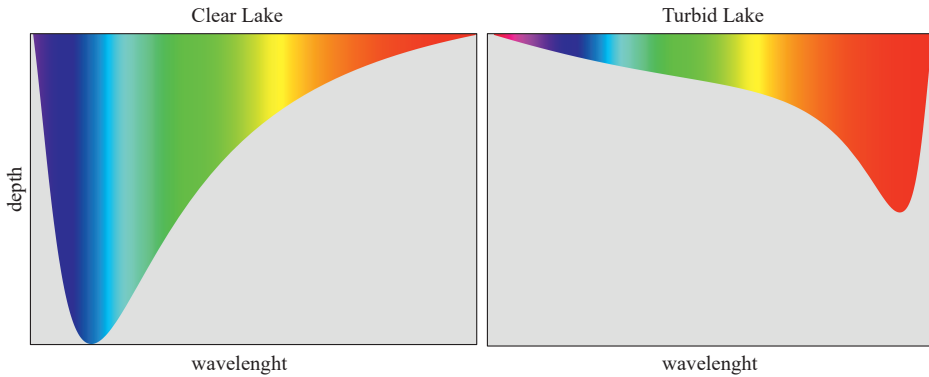
Figure 1.1. Underwater light conditions in clear and turbid waters. Light attenuation in turbid waters is much faster.

Lake Tanganyika cichlids – Lake Tanganyika, the oldest (~9-12 Ma; Cohen *et al.* 1993; 1997) and deepest of the East African Great Lakes (1470 m; Konings 2015) harbours around 250 cichlid species (Turner *et al.* 2001), highly diverse in ecology, morphology and behaviour. This diversity is suggested to be the result of adaptations in the cichlids respective niches (e.g. Clabaut *et al.* 2007). Being one of the clearest freshwater lakes in the world, the light spectrum of Lake Tanganyika results in a shift towards shorter wavelengths (i.e. blue) with increasing depth (Figure 1.1a).

Lake Victoria cichlids – About 100 000 years ago two distantly related lineages (i.e. Upper Nile and Congo lineage) are believed to have hybridized in the Lake Victoria region which provided much genetic variation for subsequent multiple adaptive radiations (Meier et *al.* 2017). Geological data suggests that the Lake completely dried out during the last glacial maximum 12 000 – 15 000 years ago (Johnson *et al.* 1996; Nagl *et al.* 2000). Subsequently, after refilling, the lake was recolonized by at least four cichlid lineages from neighbouring lakes and rivers (Seehausen *et al.* 2003; Meier *et al.* 2017). This hybrid swarm provided genetic variation that allowed together with ample novel ecological opportunities, subsequent adaptive radiation.

Hence, the Lake Victoria cichlid flock (~500 known species; Seehausen *et al.* 2002) likely evolved in less than 15 000 years (Seehausen 2006; Johnson *et al.* 2000). Lake Victoria cichlid species display a large diversity in morphology, trophic specialization (e.g. algae scraper, insectivores; Witte and van Oijen, 1990; Seehausen 1996; Bouton *et al.* 1997), as well as visual system properties (Seehausen *et al.* 2008; Wright *et al.* 2019). In Lake Victoria, most cichlids belong to the tribe of *haplochromini*. Unlike neighbouring Lake Tanganyika, Lake Victoria

has high turbidity, resulting in a shift of the light spectrum towards longer wavelengths (i.e. red) with increasing depth (Degens *et al.* 1971; Hecky 1993; Seehausen *et al.* 1997; Castillo Cajas *et al.* 2012; Wright *et al.* 2019), so that species inhabiting greater depths experience little short-wavelength light (Figure 1.1b).

Visual adaptation in cichlids – The visual system of cichlids provides opportunities to study how adaptation can contribute to speciation, as it shows extensive variation among species and populations (reviewed in Carleton *et al.* 2016) as a result of genetic adaptations and/or phenotypic plasticity (Collin and Shand 2008; Smith *et al.* 2012b; Hornsby *et al.* 2013; Dalton *et al.* 2015). Vertebrate colour vision is mediated by cone pigments that consist of an opsin protein covalently bound to a light absorbing Vitamin-A derived chromophore (Wald 1968) (Figure 1.2). Opsins contain seven transmembrane alpha helices (Figure 1.1a) surrounding a binding pocket for the chromophore. Cichlids possess seven cone *opsin* genes: three short-wavelength-sensitive opsins (*SWS1*, *SWS2a*, *SWS2b*), three medium-wavelength-sensitive opsins (*Rh2b*, *Rh2aα*, *Rh2aβ*) and one long-wavelength-sensitive opsin (*LWS*). Differences in visual sensitivity within and across species are determined by variation in the *opsin* coding sequence, which changes spectral sensitivity (reviewed in Carleton *et al.* 2016). In addition*, opsin* gene expression levels have been shown to change in response to changes in environmental light conditions in several fish species, with varying degrees of plasticity among species (e.g. bream: Shand *et al.* 2008; cichlids: Nandamuri *et al.* 2017; killifish: Fuller *et al.* 2005). *Opsin* gene expression has also been shown to be developmentally plastic (ontogenic changes) in some fish, including cichlids (Takechi and Kawamura 2005; Carleton *et al.* 2008; O´Quin *et al.* 2011; Dalton *et al.* 2015; Irazábal-González *et al.* 2022). For instance, riverine Tilapia shift from high levels of short-wavelength-sensitive *opsin* expression as larvae and juveniles to high long-wavelength-sensitive *opsin* expression as adults (Carleton *et al.* 2008). Pigment absorption patterns not only depend on opsins, but also on the type of chromophore. In particular, fish (as well as amphibians and some reptiles) use two types of chromophores, based on either Vitamin A_1 (11-cis-retinal) or Vitamin A_2 (11-cis 3,4-didehydroretinal). Marine fish and some freshwater fish possess solely A_1-derived chromophores, while most freshwater fish carry only $A_{2,}$ or A_1/A_2 mixtures (Bridges and Yoshikami 1970; Reuter *et al.* 1971; Provencio *et al.* 1992; van der Meer and Bowmaker 1995; Toyama *et al.* 2008; Morshedian *et al.* 2017). In some species, chromophore ratios are phenotypically plastic, changing with environmental and/or life-history variables, such as development, diet, season, migration or temperature (Munz and McFarland 1977; Suzuki *et al.* 1984). Switching from A_1 to A_2 chromophore type can occur within a few weeks (Munz and McFarland 1977) and results in greater sensitivity to longer wavelengths of light (i.e. towards yellow and red) (Figure 1.2b), which is important in turbid waters. Also in cichlids there are some indications for species differences in chromophore usage, with A_1-based chromophores

tending to dominate in species inhabiting clear waters (Lake Malawi cichlids; Carleton *et al.* 2000, Parry *et al.* 2005; Sugawara *et al.* 2005) while species occupying turbid waters show higher usage of A_2-based chromophores (Lake Victoria cichlids; Terai *et al.* 2006). Recently, it was found that the *cyp27c1* gene correlates with the A_1/A_2 ratio and it was found responsible for encoding the enzyme that converts A_1 into A_2 (Enright *et al.* 2015). In line with this, studies in bullfrog, lampreys and Neotropical cichlids have documented positive correlations between *cyp27c1* expression levels and A_2 proportions in cone pigments (Morshedian *et al.* 2017; Torres-Dowdall *et al.* 2017; Figure 1.2b). This suggests that *cyp27c1* expression levels can be used as a proxy for A_2 proportions.
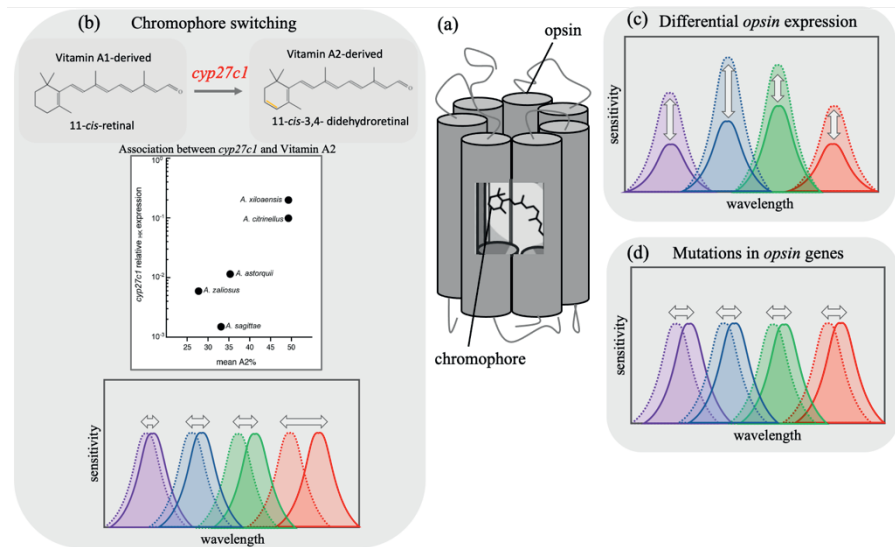

Figure 1.2. Schematic representation of the (a) visual pigment, composed of an opsin protein and a chromophore, and three mechanisms of visual tuning (b - d): (b) Chromophore switching: Conversions from Vitamin A_1 into A_2, mediated by *cyp27c1,* result in red-shifted sensitivity, with larger shifts towards long-wavelengths; (c) Differential *opsin* gene expression determines the absolute sensitivity and (d) Mutations in *opsin* genes change wavelength sensitivity. Both chromophore switching and *opsin* gene expression are environmentally plastic.

Study species – This thesis focusses on three *Haplochromini* species from Lake Victoria (**Chapters 2, 3 & 4**) and the *Lamprologini* tribe from Lake Tanganyika (**Chapter 5**). In Lake Victoria, I focus on *Paralabidochromis sauvagei* and sympatric pairs of *Pundamilia* species (Figure 1.3). *Pundamilia pundamilia* (blue phenotype; Seehausen 1996) and *Pundamilia nyererei* (red phenotype; Witte-Maas and Witte 1985) are two closely related rock-dwelling

cichlid species. Males of the species pair differ in nuptial coloration: blue males *(Pundamilia pundamilia and P. sp."pundamilia-like")* display a blue/grey coloration and red males (*Pundamilia nyererei* and *P. sp."nyererei-like"*) are yellow on the flanks and orange or red dorsally (Seehausen 1996). Females are inconspicuously coloured and exert male colour-mediated assortative mate preferences (Seehausen and Van Alphen 1997; Haesler and Seehausen 2005; Stelkens *et al*. 2008; Selz *et al* 2014). Until recently, all populations with blue males were classified as *P. pundamilia* and all populations with red males as *P. nyererei*. However, recent population genomic analyses have shown that about 12 000 years ago, the Mwanza-Gulf was first colonized by *P. pundamilia,* followed by *P. nyererei*, with admixture between the two species generating a hybrid population. This hybrid population later speciated into blue and red phenotypes as *P. sp."pundamilia-like"* and *P. sp."nyererei-like"* (Meier *et al.* 2017; Meier *et al.* 2018), that can still hybridize. In all *Pundamilia* populations considered in this thesis (except in Luanso), the blue and red phenotypes differ in ecology and tend to have different depth distributions coinciding with different photic environments: blue phenotypes are benthic insectivores and inhabit shallow waters with broad-spectrum light, while red phenotypes are insectivores/zooplanktivores and occur at greater depths, where long-wavelength light (i.e. yellow and red) dominates (Seehausen *et al.* 2008). Differences in visual environment between geographic locations and depth ranges coincide with differences in visual system properties: *Pundamilia* populations differ in the amino acid sequence of the long-wavelength sensitive opsin gene (*LWS*) and also show differences in *opsin* gene expression levels (Seehausen *et al.* 2008; Wright *et al.* 2019). *Paralabidochromis sauvagei* males have a yellow body coloration, a blue dorsal fin and a blue/grey/red caudal fin, while females are bright yellow (Seehausen 1996). *Pa. sauvagei* is an insectivore and has a relatively broad depth distribution (Seehausen 1996) and therefore encounters a range of light conditions, from broad-spectrum light in shallow waters to red-shifted conditions at the deeper end of its depth distribution. Mate choice has not been studied in *Pa. sauvagei*, but colour likely plays an important role also in this species, in line with other haplochromine cichlids (Maan and Sefc 2013). Together they provide an opportunity to test the mechanisms of sensory diversity, even between closely related species.

With 84 endemic described species, *Lamprologini* form the most species-rich and diverse cichlid lineage in Lake Tanganyika. All species are substrate spawners, with either maternal or biparental care, and show diversity in morphology and ecology, with most species occurring in shore habitats, while some inhabit open water habitats (Konings 2015). *Lamprologini* cichlids inhabit a wide range of depths: from depth range specialist (i.e. species occupying either shallow or deep water) to depth-range generalists (i.e. species distributed along a broader depth range). This species-rich tribe with multiple generalist and specialist species represents an opportunity to explore whether cichlid speciation is associated with changes in depth ranges.
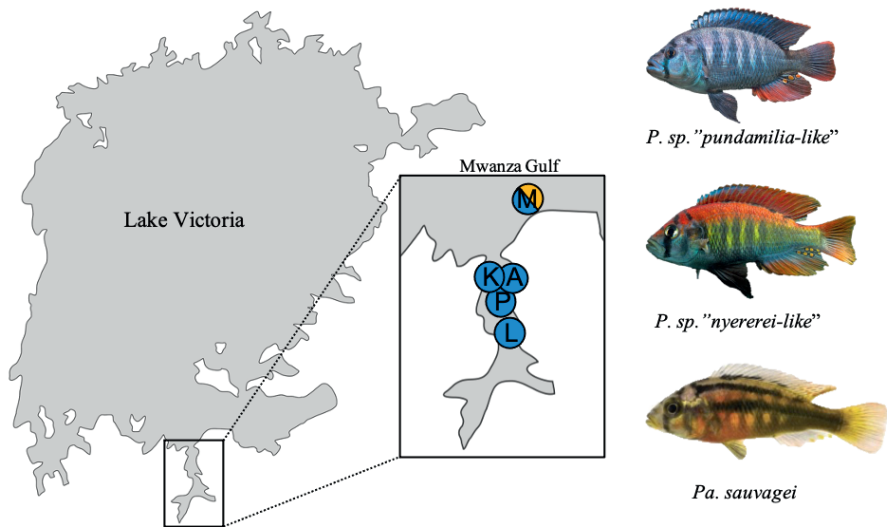
Figure 1.3. The Mwanza Gulf in southern Lake Victoria with sampling locations Anchor (A), Makobe (M), Python (P) and Kissenda (K) of both *Pundamilia* species (blue) and *Pa. sauvagei* (orange). Illustrated are *Pundamilia* males from Python island and a *Pa. sauvagei* male from Makobe island.

Thesis overview

In this thesis, I studied the mechanisms of visual diversity in the context of cichlid speciation. Specifically, I asked four questions: 1) Is there a potential contribution of chromophore usage to visual adaptation in cichlids? 2) What is the role of plasticity in cichlid visual system variation? 3) What is the correlation between visual system properties and individual behaviour? and 4) What is the impact of visual habitat segregation on cichlid speciation? To address these questions, I studied the molecular basis of visual system variation (i.e. *opsin* genes and chromophores) in wild caught (**Chapter 2**) and laboratory-reared cichlids (**Chapter 2 & 3**), investigated whether variation in visual system properties correlate with visual habitat preferences (**Chapter 4**) and explored the influence of changes in visual niches on speciation rates (**Chapter 5**) (Figure 1.4).

Contribution of opsins and chromophores to cone pigment variation across populations of Lake Victoria cichlids

While *opsin* evolution has been extensively studied in cichlid species, the contribution of other components of the visual system (e.g. chromophore usage) to adaptation remains largely unknown. In **Chapter 2**, I therefore explored the potential contribution of differential chromophore usage to visual adaptation in Lake Victoria cichlid fish. I determined whether *cyp27c1* (i.e. enzyme responsible for converting Vitamin A_1 into A_2) is expressed in wild fish and investigated its variation across multiple populations. As pigment absorption patterns not only depend on the type of chromophore, but also on *opsins* genes and their interactions, I also investigated whether variation in *cyp27c1* expression is correlated with *opsin* expression patterns. I focused on species pairs of *Pundamilia* including sympatric phenotypes, with blue and red male nuptial coloration, from five locations. Sampling locations differed in water clarity, and hence different light environments, with more turbid waters at the southern end of the sampled region (i.e. Luanso, Kissenda and Python islands) and clearer waters at the northern end (i.e. Anchor and Makobe). *Cyp27c1* was expressed at very low levels in *Pundamilia* compared to other cichlid species, suggesting that it may not be very important for visual adaptation in this species. Nonetheless, I found higher *cyp27c1* expression in populations from turbid-water than in populations from clear-water locations in the red phenotype, but not in the blue phenotype. Across populations, differences between the sympatric phenotypes in *cyp27c1* expression had a consistent relationship with species differences in *opsin* expression patterns, but the red/blue identity reversed between clear- and turbid-water locations. As a result, there was a consistent relationship between phenotype differences in *cyp27c1* expression levels and phenotype differences in *opsin* expression levels.

Visual system plasticity is differently mediated by opsin expression and chromophore composition in closely related cichlid species

The variation in chromophore usage observed in **Chapter 2** could be (partly) due to phenotypic plasticity. In **Chapter 3**, I therefore explored the contribution of developmental and environmental plasticity to different components of the visual system. I focused on the offspring of wild caught *Pundamilia* fish from Python island and *Paralabidochromis sauvagei* from Makobe island. To induce a plastic change in visual system properties, I reared the three species under two different experimental light conditions. I then quantified *cyp27c1* expression patterns and explored the association between *cyp27c1* and *opsin* expression patterns. I found differences in both *cyp27c1* and *opsin* expression patterns between the three species. However, experimental light manipulations affected the developmental trajectory of *cyp27c1* expression

in only one species (i.e. *Pa. sauvagei*). This suggests that visual system plasticity of closely related species can be differentially mediated by *opsin* expression and chromophore usage. Together these results indicate that the observed variation in *cyp27c1* expression among natural populations in **Chapter 2** largely reflects genetic differences.

Consistent preference for blue light across three cichlid species, despite differences in visual system properties and light conditions during development

In **Chapter 4**, I investigated whether the variation in visual system properties observed in **Chapter 2 & 3** correlated with visual habitat preferences. Understanding preferences for some habitats over others and the determinants of these choices are important, given that this behaviour sorts phenotypes in space and exposes organisms to new selective pressures, which might contribute to spatial isolation as well as evolutionary change (e.g. ecological speciation). In **Chapter 4**, I therefore evaluated light preferences of three Lake Victoria cichlid species differing in visual system properties, and linked these differences to a possible underlying mechanism (i.e. *opsin* expression). As in **Chapter 3**, I focused on the offspring of wild caught *Pundamilia* fish from Python island and *Pa. sauvagei* from Makobe island. I found that fish did not behave randomly with respect to the different light conditions: each of the three tested species spent most time in the blue-shifted light condition. I found only weak support for an association between *opsin* gene expression and light preference. Consequently, visual system properties do not predict habitat choice, instead these findings are in line with a general pattern observed in fish species: a preference for blue light under these experimental conditions.

A phylogenetic comparative analysis to explore the association between depth ranges and species richness in Lamprologini cichlids

In aquatic environments, speciation has been suggested to coincide with changes in water depth ranges (Recknagel *et al.* 2014; Wagner *et al.* 2014). Many biotic and abiotic factors, such as temperature, dietary resources and parasites, vary along the water column, generating depth-dependent selective regimes that may have macro-evolutionary consequences. One major factor that varies with water depth is underwater light conditions (Figure 1.1). In **Chapter 5**, I explored the relationship between changes in visual niches (i.e. depth ranges) and speciation rates. I focused on the species-rich *Lamprologini* tribe from Lake Tanganyika and classified species based on their depth distribution (i.e. visual niche): depth range specialists (i.e. species occurring in either shallow or deep waters) or depth range generalists (i.e. species with a broader depth distribution). Particularly, I explored 1) the association between

diversification rates and changes in depth range and 2) whether depth distributions change during lineage evolution, using a likelihood-based inference framework. This framework takes a phylogenetic tree and trait information to assess the interaction of an evolving trait and branching patterns, it allows trait changes during speciation and includes a procedure that avoids elevated type I errors. I did not find evidence that shifts in water depth range coincide with speciation events; instead depth ranges change along the lifetime of lineages.

Synthesis

Finally, in **Chapter 6**, I summarize my findings, integrate the results of all the chapters, discuss their implications and elaborate on directions for further research.

Visual system diversity in cichlids: behavioural, developmental and eco-evolutionary aspects
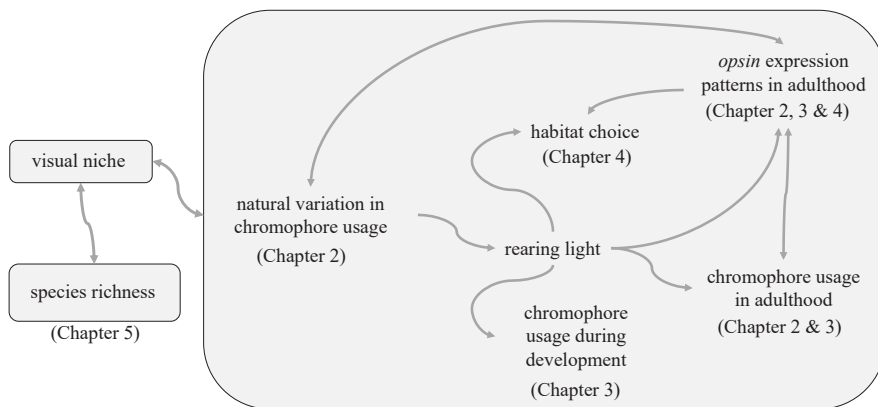

Figure 1.4. Thesis overview - Chapter 2 describes the natural variation of chromophore usage in populations of two closely related *Pundamilia* species and links it to *opsin* expression patterns. Chapter 3 explores the developmental pattern of chromophore usage and the extent of environmental plasticity across life stages in three Lake Victoria cichlid species and Chapter 4 investigates light preferences and links them to the *opsin* expression patterns in three Lake Victoria cichlid species. Chapter 5 explores the association between visual niches and diversification rates in *Lamprologini* clade from Lake Tanganyika