Summary
Speciation – the process by which new species are formed – has been the subject of intense scientific research for many years. Though evolutionary biologists have made great strides toward understanding the many intricacies of this process, numerous exciting hypotheses remain unexplored. The research contained within this thesis examines how ecological adaptation can initiate or contribute to speciation; focusing, in particular, on how it influences sexual communication and female choice.

Speciation entails the formation of barriers that prevent the exchange of genes between populations (i.e., gene flow). The local environment can play an important role in this process. Individuals and populations must be able to cope with local environmental conditions; those better adapted to the environment will better survive and reproduce. If environmental conditions vary between populations, then local adaptation may lead to population differentiation and act as a barrier to gene flow. Ecological adaptation, therefore, can be a potent force in speciation, serving as a major initiator of isolation.

Even in the presence of environmental differences, individuals from separate populations may still interbreed. This can hamper local adaptation, as favorable, population-specific combinations of genes are disrupted. However, if mating is assortative, i.e., locally adapted individuals mate only with one another, favorable combinations of genes can be maintained, allowing local adaptation and population divergence to proceed. This can create a strong association between local adaptation and non-random mating that cannot be broken by interbreeding and gene flow. The combined influence of ecological adaptation and assortative mating is well-supported; some of the strongest indications are from studies of sensory drive, where sensory abilities influence both performance in the local environment and the perception of potential mates.

The sensory drive hypothesis predicts that the sensory conditions of the local environment ‘drive’ evolution in a particular direction; sensory systems, display signals, and signaling behaviour co-evolve with the local environment. This hypothesis predicts that individuals will mate more often with partners that they can more easily detect and that mating preferences may be stronger for display signals that stand out in the local environment. Any change in the sensory systems could result in a change in preference, by making different signals more or less attractive. The local environment can also affect the signal directly; individuals possessing signals better adapted for transmission and perception in a given environment will be more successful. Both factors are important, but theory suggests that environmental effects on preferences may be more powerful in changing mating patterns and, thereby, the evolution of reproductive isolation.

Most examples of sensory drive come from aquatic animals and visual adaptation, since in water, the ambient light environment changes with increasing depth or turbidity. This pronounced and stable variation in visual conditions naturally drives aquatic species to adapt their signals and visual functioning. Aquatic environments, therefore, provide a natural laboratory to study sensory drive. Fish have provided some of the strongest examples of this; past research has implicated sensory drive in guppies, sticklebacks, killifish, swordtails, surfperch, and pygmy perch. Sensory drive has also been implicated in the rapid speciation
of the colourful cichlid fishes from Lake Victoria. Here, cichlids have radiated into as many as 500 different species (in only ~15,000 years) and male coloration and visual adaptation are thought to have played an important role. In this thesis, I experimentally test the link between vision and mate selection in Lake Victoria cichlids.

Fish, like humans, possess visual pigments in their retinas, comprised of a light-sensitive chromophore, bound to an opsin protein. There are several distinct classes of opsins; each maximally sensitive to different wavelengths of light (colour). The relative expression levels of these different opsins dictate colour vision. In many fish species (including cichlids), prior studies have shown light environment-induced changes in opsin expression, corresponding to changes in visually-mediated behaviour. I used this phenomenon as an experimental approach to explore the link between visual perception and visual behaviour in *Pundamilia* cichlids.

*Pundamilia pundamilia* and *Pundamilia nyererei* are two closely related species of rock-dwelling cichlids that co-occur at open-water and offshore rocky islands in southeastern Lake Victoria. Males of the two species are brightly coloured (*P. pundamilia* are blue, *P. nyererei* red), while females of both species are yellow/grey. At some locations, the two species have mixed and then again speciated into similar blue and red forms, known as: *P. sp. ‘pundamilia-like’* and *P. sp. ‘nyererei-like’*. At all locations, the two forms differ ecologically: the blue species lives in shallow waters, while red species extends to greater depths. Due to high turbidity in Lake Victoria, the ambient light environment shifts toward longer wavelengths with increasing depth, so the red species tend to live in an environment largely absent short-wavelength (blue) light. Male coloration is important for female mate preference in both species (females choose conspecific males based on blue vs. red coloration) and behavioural tests of visual sensitivity correspond with male colour; the red species (*P. nyererei*) is more sensitive to long wavelength (red) light and the blue species (*P. pundamilia*) is more sensitive to short wavelength (blue) light. These differences correlate with species-specific variation in visual system properties: red *P. nyererei* / *P. sp. ‘nyererei-like’* typically possess an opsin that is more red-sensitive than the corresponding opsin found in blue *P. pundamilia* / *P. sp. ‘pundamilia-like’*. Together, these factors - differences in visual environments, male coloration, female mate preferences, and visual properties - implicate sensory drive as a mechanism of speciation in *Pundamilia*.

As shown above, there have been several studies of mate choice and visual perception in *Pundamilia*. However, the observed correlation between the visual environment and species-specific visual properties and mating preferences may have come about in a number of ways. For example: visual adaptation may function as a ‘magic trait’, simultaneously affecting both ecological performance and mate choice. On the other hand, the correlation between visual properties and mate preference may have developed by indirect selection, where assortative mating among locally adapted individuals results in increased offspring fitness, thereby favouring the evolution of species-assortative mating preferences. Here, I sought to experimentally explore the mechanistic link between visual perception and reproductive isolation, testing the role of sensory drive as a cause of divergent
mating preferences between blue and red forms of *Pundamilia*. To do this, I manipulated the visual environment of both species in the laboratory, to induce changes in visual system development. I then tested the consequences of this response, by examining its influence on visually-mediated behaviour and by quantifying changes in the visual system.

In **chapter 2**, we examined how the local light environment influenced female preference for differently coloured males. We reared females of both species, as well as hybrids of the two, in shallow- and deep-mimicking light environments and then tested female mate preference for blue vs. red males. Results showed that the rearing light environment significantly influenced preference: females reared under light conditions mimicking the shallow environment of Lake Victoria preferred blue males, while females reared under light conditions mimicking the deep environment tended to prefer red males. As a result, species-assortative preferences were absent when females were reared in an ‘unnatural’ light environment (e.g. females of the blue species reared in the deep environment did not prefer blue males). This suggests that changes in visual perception can directly influence mate preference, providing behavioural support for the sensory drive hypothesis.

Our experimental light manipulations could have also influenced the male colour signal, so in **chapter 3** we asked if there were changes in male coloration. Species-specific coloration (blue vs. red) was not influenced by differential rearing, nor did coloration change when males were switched between light conditions as adults. This is in line with predictions of sensory drive: species differences in male colour signals, which are the subject of female mate choice, do not change in response to environmental conditions. This is, however, not to say that male colour is entirely fixed. We observed a small, but significant difference in green coloration; deep-reared males were greener. Perhaps, this subtle colour plasticity aids male signaling in a dark or narrow-spectrum light environment.

To establish how variation in opsin expression contributes to visual adaptation, **chapter 4** examined natural patterns of opsin expression in wild caught fish. During fieldwork in Lake Victoria, I sampled blue and red males from multiple island locations, with different light conditions, and then compared relative opsin expression between species at all locations. We found that opsin expression differed between the species, but this difference was inconsistent between islands. At two locations with clear water, the red species expressed more of the long wavelength (red) sensitive opsin (LWS) than the blue species, but at the two locations with turbid water, this pattern reversed. Prior work had already identified LWS polymorphisms, which I later confirmed in my sample of fish: the blue species (from multiple locations) share a version that differs from the form found in the red species. Thus, our results show that species differences in opsin genotype do not consistently align with species differences in opsin expression. This result not only raises questions about how opsin genotype and opsin expression together shape visual performance, but it also has implications for our light-manipulated lab population. Fish used in our experiment came from a location where, in contrast to expectation, the blue species expresses more LWS than the red species. Our light manipulation was aimed to induce correlated shifts in opsin expression and colour preference, based on the assumption that higher expression of e.g. LWS would correlate with
female preferences for red males. Given these newly observed patterns of species-specific opsin expression, this assumption may not be true for our experimental population.

In chapter 5, we returned to our lab population to specifically explore the genetic mechanisms linking visual perception to reproductive isolation. As in chapter 4, we measured the relative opsin expression of fish reared in our two light environments. We found that the light manipulations significantly influenced opsin expression; e.g. deep-reared fish expressed more of the red-sensitive pigment. Higher LWS expression also tended to correlate with female preference for red males (from chapter 2) but, as noted above, this is in opposition to the population level patterns observed in our fish (in the blue species, we observe higher LWS expression and female preference for blue males). However, the expression-preference relationship was independent of the light-induced changes in expression, so we were unable to infer a direct, causal relationship. We also genotyped the LWS opsin of females tested for preference behaviour and found that it too covaried with female preference, but only when females were tested in the shallow (full-spectrum) light environment. Together, these findings confirm that visual perception plays a role in female preference behaviour, but they fall short of establishing a causal relationship between the two. However, our inability to establish visual adaptation (i.e. relative opsin expression) as a ‘magic trait’ does not preclude the existence of such a mechanism. It is possible that other visual properties (e.g. opsin genotype) serve this function.

Visual adaptation to different light environments implies that a mismatch between the visual system and the local light environment should result in decreased performance in visually mediated tasks. In chapter 6, I tested this hypothesis. I examined the foraging performance of fish tested in their ‘natural’ (blue fish in shallow, red fish in deep) and ‘unnatural’ (blue fish in deep, red fish in shallow) light environment and found that ‘natural’ testing resulted in higher prey capture. This suggests that each species is visually adapted to maximize foraging performance in their respective environment. To establish a causal link between visual perception and performance, I also examined the effect of our rearing light manipulations. I found weaker effects: fish reared in the deep light environment caught slightly more prey than their shallow-reared counterparts. This suggests that environment-induced changes in opsin expression (as observed in chapter 5) do not strongly affect visual performance. Together, the results of chapter 5 and 6 highlights the importance of species-specific (visual) factors, presumably genetic, for visually-mediated tasks.

This thesis used an integrative approach to examine the role of visual adaptation in speciation. We used behavioural assays, colour analysis, visual system characterization, and field surveys to test predictions of the sensory drive hypothesis in Pundamilia cichlids. Our light manipulations affected both visually-mediated behaviour (female mate preference) and visual system development (relative opsin expression), but we were unable to establish a causal relationship between the two. We also genotyped females for variation in the LWS opsin and found that it too covaried with female preference behaviour. In wild populations, we found that species-specific patterns of opsin expression do not consistently align with opsin genotype, male colour, and female preference. Together, these results suggest that
Summary

opsin genotype, rather than expression, may act as the causal link between colour perception and mate preference. Future research will benefit from exploring this link further, making use of the available techniques to manipulate the opsin genotype.