Early learning and speciation : the effects of early experience on sexual and aggressive behaviour in Lake Victoria cichlid fish

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Chapter 1

General introduction and summary

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General Introduction

Development of species recognition lies at the heart and base of behavioural biology, both conceptually and historically. Konrad Lorenz followed by the goslings is a classical image that is familiar to many people the world over. His work clearly showed how geese and many other animals develop recognition of their parents and subsequently of their own species, or in this case of Konrad Lorenz, through learning at a very young age (Lorenz 1937). The fascination of how animals grow up and develop to show the complex behaviour that they do has inspired many researchers. The enormous variety of behaviour in the animal kingdom invites questions of how and why such diversity has evolved. Lorenz, among others, examined species differences in behaviour. Comparing species points us to differences, and, equally important, to similarities between them. It is these patterns that help us understand the relatedness and evolution of those species and their specific characters. This approach was also advocated by Niko Tinbergen, another founding father of behavioural biology. He pointed out that behaviour can be studied to answer questions at different levels. Not only can behaviour be studied to find out how it develops and how it is internally organised within an individual, but behaviour can also be an adaptation and evolve, like all other traits of an organism, and thus lead to differences between species (Tinbergen 1963). Behaviour is in that respect no different from any other trait of animals. Behaviour is special however, because it directly influences how individuals interact and it is the interaction between individuals that enables them to successfully hold territories, mate, and raise their offspring. Behaviour holds the key to reproductive success, and therefore can have profound influences on evolutionary processes. This thesis deals with the study of development of species recognition in Lake Victoria cichlid fish and I place this in the context of their speciation.

Development of species recognition is one of the best studied processes in behavioural biology, though the vast majority of those studies have been on bird species. Species recognition may develop through imprinting, such as in Lorenz's goslings. Imprinting, often also referred to as early learning, is a form of learning that occurs early in life. Goslings, for instance, learn the features of something they see soon after hatching, most likely either their mother or their siblings. They use these features to recognise whom to follow and from which individuals to accept that they approach and whom to take food from. What the gosling has learnt at an early stage of its life is thus later used in social contexts. When imprinted information influences interactions with an individual's parents and or its siblings, it is called filial imprinting. Learned information may also have an effect on mate selection at a later age, known as 'sexual imprinting', or on rival recognition in territorial defence. Filial and sexual imprinting may be a similar form of learning, but they often don't coincide, and hence are probably two separate processes (Bateson 1979). Both filial and sexual imprinting are widespread in birds, in which these processes have been studied extensively (ten Cate & Vos 1999; Bolhuis 1991). More recently, sexual imprinting has also been found in mammalian species (Kendrick et al. 1998), including in humans (Bereczkei et al. 2004). The widespread occurrence of imprinting indicates that it may be the standard in several animal groups. Song learning in birds is another very well studied example of development of behaviour, and shares the characteristics with imprinting that it happens early in life, and is often learnt from a particular individual and is of prominent importance for species recognition. It therefore has similarities with learning processes involved in imprinting. An important difference, however, is that in song learning, individuals learn a trait, rather than a preference for a trait. However, learning a preference for specific songs by many female songbirds at an early stage in life shares many of the features of imprinting (Riebel 2003a).

Early studies on imprinting suggested the existence of a critical period for irreversible learning. Later studies have shown that such periods are not so clear cut, nor are they always irreversible (reviews in Bateson 1979; Immelmann 1975). Due to these findings, some people have started to use the term 'early learning' in stead of imprinting, meaning just the occurrence of learning at an early age, without any claims to sensitive periods and permanent effects. I use both terms here interchangeably. While I prefer the term early learning because it is uncontroversial, many readers may be more familiar with imprinting, which is why I also use this term.

Species recognition and mate preferences may also develop through other mechanisms than imprinting. Other types of learning may play a role, for instance, species recognition may develop through interactions with conspecific and heterospecific individuals at a later age (Schlupp & Ryan 1997; Magurran & Ramnarine 2004; Dukas 2004). Genes may play a role in the formation of species specific preferences. Genetic differences between species have been indicated to mediate preferences for own species phenotypes over heterospecific ones in insect species, such as crickets (Shaw 2000; Ritchie 2000), and drosophila (see overview in Coyne & Orr 2004). Genes by learning interactions may also occur when unlearnt predispositions that can limit and canalize the effects of experience (e.g. ten Cate 1994). An example of such a genetic by learning interaction is song learning in white crowned sparrows. Although song sparrows learn the song they sing, their genetic background biases which song type they will learn (Marler 1970).

Imprinting and speciation

Speciation has been a major topic in evolutionary biology since Darwin published his work in 1859 (Darwin 1859). During the modern synthesis of evolutionary biology, the ideas of Darwin were connected with knowledge of heritability, genes, and mutation (e.g. Mayr 1942). Speciation became a major topic of theory and subsequent empirical investigation. Species recognition was one of the major contributions of behavioural biology (called ethology at that time) to this field.

Interest in this classical study subject has recently regained attention in the context of speciation (Irwin & Price 1999; Laland 1994; Servedio et al. in press; ten Cate 2000; ten Cate & Vos 1999; Albert 2005; Beltman & Metz 2005; Grant & Grant 1997). Because of the genetic, and therefore phenotypic similarity between parent and offspring, imprinting has the interesting property of ensuring a relatively good match of social and sexual preferences and own phenotype (Albert 2005; Irwin & Price 1999; Laland 1994; ten Cate & Vos 1999). This means that individuals will interact and mate with individuals that look like, smell like, or sound like themselves. This phenotype assortative behaviour is an important prerequisite for speciation, because it will mediate reproductive isolation. During speciation, two populations of a species diverge and mating will take place within each diverged population. When they are geographically isolated, the tendency to interact with individuals with similar phenotypes is not under selection. However, when the two populations were never separated by distance (sympatric or parapatric), or later spread their range of occurrence to overlap again (secondary contact), species and mate recognition mechanisms are under selection to become more specific (Dobzhansky 1940; Blair 1955; Dieckmann & Doebeli 1999; Kondrashov & Kondrashov 1999; Servedio 2004; van Doorn et al. 2004). This is because of the negative effects of mating with other species and producing hybrid offspring. Individuals should prefer to mate with individuals that have a similar phenotype as themselves. Thus, not only should preferences in a population change, they should also become linked with the newly arisen traits. The linking of any two traits is difficult because recombination during meiosis breaks up combinations that may occur on particular chromosomes. Therefore, the combinations of those traits need not be heritable. This linking becomes more and more difficult when there are more genes involved (Felsenstein 1981; Kirkpatrick & Ravigne 2002). Linkage between traits and preferences can only build up with assortative mating, causing linkage disequilibrium. Theoretical studies on speciation therefore have concluded that for speciation to be likely to happen, mate preferences should be mediated by only one or very few genes (Arnegard & Kondrashov 2004; Dieckmann & Doebeli 1999; Kirkpatrick & Ravigne 2002; Kondrashov & Shpak 1998; Servedio 2000; van Doorn et al. 2004). Sexual imprinting may provide a situation in which such problematic build up of linkage disequilibrium is not necessary, because the parents that individuals imprint on will look very similar to themselves, and thus they will develop preferences for their own type. Imprinting may thus solve two issues indicated as problematic in speciation theory: it will immediately provide preferences for newly arisen phenotypes (Irwin & Price 1999; ten Cate & Vos 1999), and individuals will have preferences for phenotypes that are very similar to that of themselves (Albert 2005; Laland 1994; ten Cate & Vos 1999).

Cichlid speciation

Theoretical predictions are made to be tested. Interesting test cases for the role of early experience in speciation are the many colourful species of haplochromine cichlids from the East African lakes. Haplochromine cichlids are mouth brooding inhabitants of the East African lakes and rivers, and comprise the endemic species flocks of the Lakes Malawi, Victoria and part of Lake Tanganiyka. Each of these lakes (Lake Victoria, Lake Tanganyika and Lake Malawi) harbour species flocks that are endemic to that lake, and together they contain at least 1100 species (Turner et al. 2001). Lake Victoria and Lake Malawi are each inhabited by single species flocks, whereby all the haplochromine species within a lake have a monophyletic origin (Verheyen et al. 2003; Salzburger & Meyer 2004; Seehausen et al. 2003). They are a diverse group in both ecology and nuptial colouration. Their species numbers and large diversity make them a model system for speciation biology (Kocher 2004; Kornfield & Smith 2000; Seehausen 2006a). Research on their speciation history has yielded many different hypotheses, including virtually all combinations between possible geographical settings and selection pressures for divergence (Seehausen 2006a; Salzburger & Meyer 2004). Theoretically the least likely speciation mode that was proposed for several species pairs is sympatric speciation driven by sexual selection. This was proposed based on their minimal ecological divergence, fully viable hybrids in the laboratory, nested population ranges and maximal nuptial colouration differences (Seehausen 2000; Kornfield & Smith 2000; Knight et al. 1998). This hypothesis about their speciation is not without controversy, and has helped fuel theoretical investigations into its possibility and likeliness, which at the same time identified the crucial factors in such a scenario. The alternative hypotheses for the speciation of those pairs include sympatric speciation driven by ecological divergence, and allopatric speciation driven by either natural or sexual selection. These speciation scenarios have in common that at some point they would have to assume reproductive isolation, in the face of sympatric conditions. In the laboratory, haplochromine cichlid species can interbreed, and hybrid infertility has not yet been observed (Van der Sluijs et al. in prep; Seehausen 2004). Thus, haplochromine cichlids do not appear to have built up genetic incompatibilities between species which would disable them from interbreeding. This means that apparently reproductive isolation is fully mediated by mate choice. More over, the high species numbers suggest a propensity for reproductive isolation by mate choice. Sexual imprinting might be a mechanism for promoting their assortative mating.

Cichlid breeding habits may be conducive to imprinting, because all haplochromine cichlids show maternal care behaviour (Salzburger et al. 2005). Males hold and defend territories, and court females. When a female approaches the displaying males, the male may lead her to the centre of his territory, where spawning may take place subsequently (Baerends & Baerends-van Roon 1950). This occurs while female and male are circling each other. A female will lay an egg, after which the male quickly fertilizes it before the female takes the egg into her mouth. Broods consist of various amounts of eggs, depending on the size of the female, and up to 4 males may fertilize eggs in one brood (Maan et al. 2004). After fertilization, the female leaves the male territory and keeps the eggs in her mouth until they hatch. This may last up to 4 weeks. After hatching the fry leave the mouth of their mother and start foraging independently. However, the mother will protect her brood for a while longer, about two weeks, and may take her young back into her mouth with approaching danger. This prolonged brood care provides the young with an opportunity to imprint on her phenotype.

Earlier work on cichlid imprinting

Studies on sexual imprinting in cichlids have been conducted before, on two species: *Cichlasoma nigrofasciatum* and *Astotilapia burtoni* (then called *Haplochromis burtoni*). These have yielded mixed results. *C. nigrofasciatum* is a Middle American cichlid, not a haplochromine, which shows biparental care for its substrate spawned broods. This species has two distinct colour morphs, one of which is rare, and these show assortative mating in nature. Several experiments were done to investigate the role of learning on morph assortative behaviour, by several people, in several setups (Weber & Weber 1976; Siepen & Crapon de Caprona 1986; Fernö & Sjölander 1976; Barlow 1992). However, the effects of early learning in *C. nigrofasciatum,* if any, are often obscured by dominance effects (Weber & Weber 1976; Fernö & Sjölander 1976). The removal of such dominance factors in later experiments by George Barlow, let him to conclude that the effect of early experience on mate preferences may be real, but weak and cannot explain the observed assortative mating in the field (Barlow 1992).

Astatotilapia burtoni is a mouth brooding cichlid from Lake Tanganyika, a haplochromine cichlid, but not part of the species flocks in this lake. It was tested for effects of siblings on male aggression biases, male mate choice, female mate choice and female aggression biases by Crapon de Caprona (1982). Young of these fish grew up with either conspecific siblings or foster siblings of *C. nigrofasciatum.* The conclusion from this study was that both males and females learnt their recognition of males from their experience with their siblings. However, the behavioural displays of the females towards males strongly suggest that they showed aggressive behaviour, while courtship behaviour was virtually absent, and thus did not recognise the males as potential mates. Also, the recognition of conspecific females (by both sexes) was not learnt from experience with siblings. Fernald (1980) found that male *A. burtoni* showed recognition of conspecific males as competitors without any prior social experience, strongly suggesting a non-learnt background for this behaviour. Though this may seem in contrast to the findings of Crapon de Caprona, a non-learnt background for species recognition may be additive to or overruled by experience. To conclude: The importance of imprinting for mate choice in cichlids has thus far remained unclear.

Male rival recognition and coexistence

Sympatric coexistence of closely related species such as that of cichlids does not only require reproductive isolation, it also requires negative frequency dependent selection (van Doorn et al. 2004; Rueffler et al. 2006). What keeps one of the species from out-competing the other species? Even if they are reproductively isolated and equally adapted to their habitat, theory predicts that chance fluctuations in population size would in time eliminate one of the species. Sources of such negative frequency dependent selection may be forms of natural selection, for instance when competition for food drives the evolution of specialists (reviewed in Rueffler et al. 2006). Most theoretical investigations of speciation and sympatric coexistence have incorporated such natural selection. The apparent lack of substantial ecological divergence of some species is a puzzling situation, which may theoretically be solved with negative frequency dependent sexual selection (van Doorn et al. 2004). Frequency dependent inter sexual selection (i.e. female choice) may be hard to envision in promoting sympatric speciation, but intra sexual selection, or male-male competition, could yield such effects (Seehausen & Schluter 2004; van Doorn et al. 2004; Mikami et al. 2004). When males compete selectively with other males that are of a similar phenotype as themselves, rare phenotypes would have fewer aggressive encounters than the common phenotype and therefore incur lower costs to obtain and maintain a territory. The reduced male-male competition for rare phenotypes should then result in increased mating opportunities. This may be through more investment in courtship or by being preferred by females, for instance because they are in better condition after less fighting. Those increased mating opportunities should be with conspecific females, in order to maintain reproductive isolation. A rare-male advantage arises from strictly assortative male-male interactions, which are proximately mediated by the development of species specific rival recognition. When the development of species recognition is particularly geared towards producing assortative male-male interactions, i.e. it is not susceptible to fluctuations in the distribution of phenotypes in the population, frequency dependence effects could occur. The ontogeny of rival recognition may be mediated by imprinting, similarly to how imprinting may mediate mate recognition (Hansen & Slagsvold 2003). Thus, rival imprinting on the maternal phenotype may, like sexual imprinting by females on the maternal phenotype, provide males with assortative rival recognition.

Cichlid fish show species assortative aggression, when challenged with a simultaneous intrusion of two males: one of their own species and one of their sister species, they are more aggressive to the intruder of their own species (Dijkstra et al. 2006b; Dijkstra et al. 2007). Their territories also often border on that of a male of another species, a pattern that would be expected with frequency dependent male-male competition (Seehausen & Schluter 2004). We do not know, however, how males may actually benefit from a reduced amount of received aggression, and if it may increase their fitness, keeping this rare male hypothesis just that for the moment: a hypothesis. Still, the study of the development of male assortative aggressive interactions will help us understand whether it may yield negative frequency dependent selection at all and possibly at what stages in speciation.

The model species

In this thesis I describe experiments that test for the effects of early experience on the behaviour of Lake Victoria cichlid species in the contexts of mate choice and male territorial interactions. Is early learning involved in the development of species recognition? The model species I used in this thesis are two pairs of closely related cichlid species: *Pundamilia pundamilia* (Seehausen et al. 1998) and *Pundamilia nyererei* (Witte-Maas & Witte 1985), and *Mbipia mibpi* and *Mbipia lutea* (both Seehausen et al. 1998). Both pairs occur sympatrically and both pairs appear very similar in ecology and breeding behaviour. They do differ in male nuptial coloration: blue and red for the respective *Pundamilia* species, and black and yellow for the *Mbipi* species, and they differ slightly in their distribution over the water column (Seehausen et al. 1998). Still, the species of each pair are fully within cruising range of each other, and they have often been observed in the same places by scuba observations and caught in the same nets. They therefore have every opportunity to interbreed. Nevertheless, they are reproductively isolated in clear water populations. The populations used in this thesis are from Makobe Island, which is a rocky island about five km from the shore in the Tanzanian side of Lake Victoria (Bouton et al. 1997). At this relatively clear water location, the species do not interbreed, as no intermediate phenotypes have ever been observed here (Seehausen 2006b). Brood care in these species is similar to all other cichlids in Lake Victoria: after fertilization, the female hatches the eggs in her mouth, and the male has no further involvement. Hatching takes about 21 days in both genera. The mother also takes up her fry after they were release from the mouth. For the *Pundamilia* species the description mentions up to seven days, but in the laboratory facilities in Leiden, I have seen it last up to 14 days. Females of the *Mbipia* genus are known to take their fry back up to day 17 after release (Seehausen et al. 1998). What happens to the fry after their mother leaves them is not known. I have observed groups of same size fry in shallow water, and shoals of sub adult individuals have been observed (Seehausen et al. 1998). Sexual maturity is usually indicated in size of 50% maturity, and does not indicate the age of the fish. The growing pace may differ with conditions, and may thus be different between nature and the laboratory. In the Leiden laboratory, males and females have been observed to be sexually mature at 10 months in all species. *P. pundamilia* and *P. nyererei* are becoming a model species pair in the research on sexual selection and speciation in Lake Victoria cichlids. Previous studies have shown that females prefer males of their own species (Seehausen 1997); that this preference depends in part on the differences in nuptial coloration between the two species (Seehausen & van Alphen 1998), and that *P. nyererei* females prefer to mate with brighter coloured males (Maan et al. 2004), which appear to be in better condition also (Maan et al. 2006b). In addition, the perception of the colours red and blue appear to be different between females of the two species (Maan et al. 2006a). These findings support the hypothesis of sexual selection through female choice, which may drive the divergence of male nuptial

Figure 1. A *P.pundamilia* female providing brood care to newly hatched fry. Photos: J. van Heusden

colouration. However, sexual selection for male nuptial coloration (through a 'good genes' scenario or a sensory bias scenario or both) may not be able to provide reproductive isolation. Haesler & Seehausen (2005) have studied female choice in hybrid females of these species, concluding that laboratory bred hybrid females loose their preferences for either species. When hybrid individuals show intermediate traits, this can be a strong indication for a genetic background for species differences. However, a lack of preferences need not indicate intermediate preferences. It may for instance also indicate that hybrid females are not choosy. Absence of evidence in this experiment for learning effects is therefore not evidence of its absence.

Outline of this thesis

In this thesis I ask the following questions: Does early learning mediate own species recognition in Lake Victoria cichlid species, both with respect to mate preferences as well as for rival recognition? The maternal care in haplochromine cichlids provides the opportunity for early learning, but do the young cichlids take this opportunity to learn? And, if so, can this promote reproductive isolation under sympatric conditions? These questions are addressed in four chapters, in which I explore early learning in the context of sympatric speciation with mathematical models, and through experiments with individuals of the species pairs I described above. I test whether species assortative female mate choice, male mate choice and male-male aggression is affected by early experience.

In chapter two the intuitive and verbal models of the role of imprinting in sympatric speciation are explored with mathematical models. Does it really promote reproductive isolation as well as we are inclined to think? This question is approached by comparing models in which females imprint with several other ways in which animals may come to mate assortatively. Although in recent years many models have addressed the question of whether the evolution of assortative mating in sympatric conditions is possible, they simplified the mechanisms of female mate choice. Although this is in some way always inevitable, and in addition it is not always clear if there are general mate choice rules (Kirkpatrick et al. 2006), some simplifications may have a large impact. In some models, females simply match their own phenotype to that of their mate, which is called self-referent phenotype matching (Dieckmann & Doebeli 1999; Kondrashov & Kondrashov 1999). In others models, females possess 'preference genes' that determine which male phenotype they prefer (Kirkpatrick 1982). I compare these two modes of female choice with various types of sexual imprinting. The effectiveness of different mate-choice behaviour in causing divergence in male traits is examined under simple deterministic one-locus population genetic models as well as under polygenic, individual-based simulations based on the models of Dieckmann and Doebeli (1999). I find that the inheritance mechanism of mate choice can have a large effect on the ease of sympatric speciation. When females imprint on their mothers, the outcome of the model is similar to phenotype matching, where speciation can occur fairly easily. Surprisingly, when females imprint on their fathers, speciation becomes considerably less likely. This is because male reproductive success is dependent on the females that choose them, which may cause considerable positive frequency dependent selection, counteracting the formation of two species. When females imprint obliquely (i.e. on a phenotype she may encounter in the population at large), speciation becomes impossible. Finally, when females rely on preference genes, male trait evolution occurs easily, but the correlation between trait and preference can be weak, and interpreting these results as speciation may be dubious.

The third and the fourth chapter are part of the same experiment. I designed a cross-fostering experiment to test for sexual imprinting in *Pundamilia pundamilia* and *P. nyererei*. For this I exchanged the eggs between brooding females. This was done both between females of the two different species as well as between females of the same species. About 50% of the times the females accepted their foster brood after which they were allowed to raise their broods as normal.

In chapter three I describe an experiment in which I tested the daughters raised under this cross-fostering regime for their mate preferences when they were sexually mature. Females could interact freely with two males, one of each species, and I observed their courtship behaviour. The adult females raised by heterospecific mothers also preferred heterospecific males in mating trials, and their preference was significantly different from the females raised by conspecific females. This shows that female mate preferences are affected by early learning about their mothers' phenotype. Mate preferences mediate assortative mating, and thus reproductive isolation. Sexual imprinting in cichlids may thus promote reproductive isolation between diverging phenotypes without accompanying genetic preference evolution. Combined with the conclusions from chapter two, I suggest that the existence of this learning mechanism may have been critical to the evolution of the remarkably high species diversity of the East African cichlids.

The fourth chapter deals with the effects of cross-fostering on male behaviour. The ability to recognise conspecifics in contexts of both mate choice and territorial defence may have large effects on a male's fitness. Understanding the development of male assortative behaviour may shed light on how species assortative behaviour evolves and how it may influence reproductive isolation, like for female mate preferences. I test whether early learning influences male mate preferences and male – male aggression biases. Males were tested for their aggression bias as well as for their mate preferences in two-way choice tests. Males cross-fostered with conspecific and heterospecific foster mothers selectively directed their aggression towards conspecific intruders. The cross-fostering treatment also did not affect male mate preferences. These results are in striking contrast with the finding that females show a sexual preference for males of the foster species. I conclude that males of these species do not use early learning for the development of species recognition in these contexts. These results are in striking contrast with the finding that females show a sexual preference for males of the foster species. I did, however, find an indication that experience in territorial contexts increases assortative male-male aggression. Since we tested each male for both contexts, we tested whether the degrees of assortativeness of the displays in the two contexts were similar, but we did not find any indication for such a relationship.

In the fifth chapter I explore imprinting in a second species pair, *Mbipia mbipi* and *M.lutea*, with the aim of replicating the results found in the two *Pundamilia* species, but also extending the experiment in order to disentangle the effect of experience with mother and experience with siblings. I thus performed another interspecific cross-fostering experiment, but this time also raised mixed broods by swapping either only a few eggs or most, but not all, of the eggs. Whereas in the *Pundamilia* cross-fostering experiment females and males were not allowed extensive interaction after the first signs of sexual maturity, the broods in this experiment were left intact until everyone was sexually mature. I tested the effect of experience with the phenotype of the mother and that of the siblings on female mate preferences and male aggression biases. Like in chapter three, I demonstrate that female mate preferences are strongly influenced by learning about their mothers' phenotype. To this, this study adds that female mate preferences are not affected by experience with their siblings, despite ample opportunity for interactions. Male aggression biases, in contrast, are affected by experience with siblings but not by learning about their mothers' phenotype. These results thus replicate the findings of the *Pundamilia* experiment, indicating that the female imprinting behaviour may be shared among the closely related haplochromine cichlids of Lake Victoria. They also confirm the sex difference in effects of early learning in males and females for aggression and mate choice respectively, as found in the *Pundamilia* cross fostering experiment. Because of the effect siblings may have on male-male aggression biases; I suggest that the development of assortative behaviour of females, but not of males, may create favourable conditions for sympatric speciation in Lake Victoria cichlids.

Summary

In this thesis, I asked whether the species assortative mate preferences of Lake Victoria cichlid fish are mediated by early learning about the maternal phenotype, and whether species assortative male-male aggression is mediated by learning about the maternal phenotype or experience with siblings. Also, I explored whether sexual imprinting on the maternal phenotype may promote reproductive isolation in sympatric speciation.

I have found that young female cichlid fish are affected by experience with their mothers' phenotype in their later mate preferences. I conclude this from two cross-fostering experiments, with two different species pairs. The similarity of this effect between the two experiments indicates that this behaviour may be present in more haplochromine species, widening the implications of this finding for our understanding of species richness in Lake Victoria cichlids. Moreover, the similarity of the results shows that the *Pundamilia* genus may indeed be a representative model system for the 23 genera in the Lake Victoria cichlid flock, which holds over 500 species (Greenwood 1980; Seehausen et al. 1998).

Maternal imprinting proved to be a mechanism favourable for sympatric speciation in a mathematical comparison of female preference development. This indicates that the propensity that the Lake Victoria cichlid fish appear to have for assortative mating, either after sympatric speciation or allopatric speciation, may be fuelled by learning.

The behaviour of the males, in contrast, was not affected by learning about their mothers' phenotype, while in the second cross-fostering experiment I found that male-male interactions were influenced by experience with siblings. This finding resembles that of the experiment of Crapon-de Caprona (1982) with *A. burtoni*. In nature, males may either learn about the phenotype of their siblings or about males in the larger population. An individuals' relatedness with its siblings is already substantially lower than with its mother (especially if several fathers may fertilize eggs in one brood) and of course the relatedness with males from the population at large is dramatically lower. Male behaviour, therefore, may be less prone to become assortative in a sympatric speciation scenario, and may therefore not be able to yield negative frequency dependent selection necessary for sympatric speciation.

What may have been learnt?

Both males and females thus learn about the phenotypes of other fish, be it about their mother during maternal care or about others at a later stage. The answer to one question raises the next question: what do they learn about? From these experiments it is not possible to disentangle what they may have learnt, i.e. have they learnt about visual features, olfactory cues, or a combination of those? In chapter five, I describe why it may be unlikely that the young fish learn about visual aspects of their mothers' phenotype while hatching in her mouth (there's probably not much light there, and they see the inside of her mouth more than anything else). During the mothers' protection of her brood, they may learn about her visual characteristics. In chapter three I show that females of the two *Pundamilia* species also show differences in coloration, but to a much lesser extent than the males, and the question remains if the young are able to pick up on those cues. Although filial imprinting and sexual imprinting may be separate processes, also in fish, cues that are learnt during filial imprinting may be available for sexual imprinting as well. Filial imprinting experiments on visual cues have been done, but primarily with substrate brooders. Baerends and Baerends-van Rhoon (1950), in a summary of results of filial imprinting experiments by themselves and others, conclude that there are large species differences in the extent to which visual characteristics are learnt by the fry. Whereas the young of some species are affected by experience, others do not seem to be at all. It may thus be possible for young of cichlid fish to learn about their parents' visual characteristics at a very young age, but these experiments were conducted on Central American cichlids, which show considerable differences in breeding behaviour from the East African haplochromine cichlids. This behaviour thus may or may not be shared with haplochromine cichlids.

If we are uncertain about the learning of visual aspects of parents by the young cichlids, we know even less about olfactory learning. However, some indications that this may be a possibility comes from the work of Kühme (1963), who found that young from the West African substrate brooder *Hemichromis bimaculatus* recognise the scent of their siblings already at 1 day after hatching. The parents, in turn, also recognise the scent of their own brood, even when given the choice of several broods of other conspecific pairs. Whether learnt or not, olfactory mate choice cues are used in choosing conspecific mates by females of two Lake Malawi cichlid species (Plenderleith et al. 2005). Whether this is also the case for Lake Victoria cichlids remains to be tested.

Finally, the observation of hybridization in turbid water has been explained by the inability of females to see species differences in coloration, suggesting that females use visual cues over olfactory cues (Seehausen et al. 1997). However, turbid water may also obscure differences in olfactory cues. Apart from the neutralisation of olfactory cues by chemical reactions (Fisher et al. 2006), the signal to noise ratio of fish smell in turbid water may be considerably worse than in clear water. Muddy water may just smell of mud. Turbid water thus may obscure both visual and olfactory signalling.

Why is it important to distinguish what young learn about their mother? One reason is that what young learn about may determine what the crucial differences are for reproductive isolation to occur. Only when the traits that are imprinted on are different enough between males of the two incipient species, it is possible for females to discriminate between the two phenotypes, with assortative mate choice as the result. Another reason is that knowing what young learn may point us to the sequence of events in a speciation process. If young learn about the colour of their mother, this may tie in directly with other possible selection mechanisms acting on male nuptial coloration, such as sensory biases (Maan et al. 2006a). If young learn about olfactory cues, then the link with nuptial coloration may be less direct, and this would change the conditions under which imprinting may promote sympatric speciation.

Imprinting and directional selection

Another question that comes to mind is what the role of imprinting is in the initial phase of speciation. Early learning may provide good conditions for reproductive isolation when variation is already available in the population, but how does it interact with creating that variation? Some accounts of the role of imprinting in speciation, through both verbal models and mathematical models, indicated that imprinting may be conservative, in the sense that it would not promote diversification, but at most maintain it or even counteract it (O'Donald 1960; Seiger 1967; Albert 2005). Even though this may not necessarily be true (Aoki et al. 2001; Kalmus & Smith 1966; Laland 1994; Weary et al. 1993), I can see a possible scenario in which the hypothetical conservative nature of imprinting at least does not counteract directional selection. This scenario involves mate choice decisions on multiple cues (which is common, see review Candolin 2003), where some of those cues are imprinted on, while others are not. Sexual selection through preferences that are not mediated by imprinting may generate initial variation. Imprinting will then be neutral when the cues that young imprint on are still similar between the diverging phenotypes. There should however, be a functional link between the different mate choice cues, making sure that differences in imprinted cues indirectly follow suit with those that are not imprinted on. Imprinting may then kick in later, as it were, and take care of reproductive isolation. In the instance of the *Pundamilia* speciation, assume that imprinting occurs on olfactory cues, while females also select males on the visibility of their colouration in the available light spectrum. Since this visibility is different at different depths (red is more visible at greater depths in Lake Victoria than blue, while blue is better in shallow water), this may select on the hue of nuptial coloration: for deeper swimming males to be red and shallow swimming males to be blue (Maan et al. 2006a). Brightness adds to the visibility of these males, which may then select the redder, deeper swimming males to be more bright red. Coloration may be linked to olfactory cues in the following way. Redness is achieved by the investment of carotenoids, which are also used in the immune system (Maan et al. 2006b). Carotenoids are only obtained through food, and redness and immune-competence thus trade off. The brightness of a male may therefore depend on both its efficiency in feeding and the composition of its immune system, also because the parasite community at deeper water locations may be different than at shallow water locations. Females thus indirectly select on these traits by choosing the brightest males (Maan et al. 2006b; Maan et al. 2004). The immune system, and the type of food an individual eats, influences olfactory cues (Zavazava & Eggert 1997), allowing the build up of an olfactory signature of red versus blue coloration. Thus the selection for brighter, more visible males could result in differences in olfactory cues, which are expressed in females also, and are thus available to the imprinting young. These differences are only picked up by the (imprinted) choosing females once the initial divergent selection has already started. The directional selection for brighter males which starts divergence of phenotypes may not necessarily result in assortative mating, because the attractiveness of a male is not dependent on the traits of the female, but rather on the depth of where male and female meet. Imprinting on olfactory cues may ensure assortative mating, and subsequently reproductive isolation, because a females' mother will express the same olfactory cues as males. The possibly conservative nature of sexual imprinting thus may take care of reproductive isolation, while not counteracting sexual selection that generates trait variation. Yet, the effect of imprinting on the generation of trait variation, both directly and indirectly, is poorly understood and invites further investigation.

Imprinting is indeed a wide spread phenomenon, and still very much alive as a research topic. In the last few years several studies, including the ones in this thesis, have shown early learning to mediate species assortative behaviour in fish (Engeszer et al. 2004; Walling subm.), firmly extending the known occurrence of imprinting to this taxon of animals as well. The appreciation of the implications of learning on evolutionary processes is also starting to gain momentum and in general, conclusions are that learning is a phenomenon not to be neglected. Which is a suggestion scientists can hardly ignore, can they?