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

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Machteld Verzijden

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Machteld Verzijden

Chapter 1

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

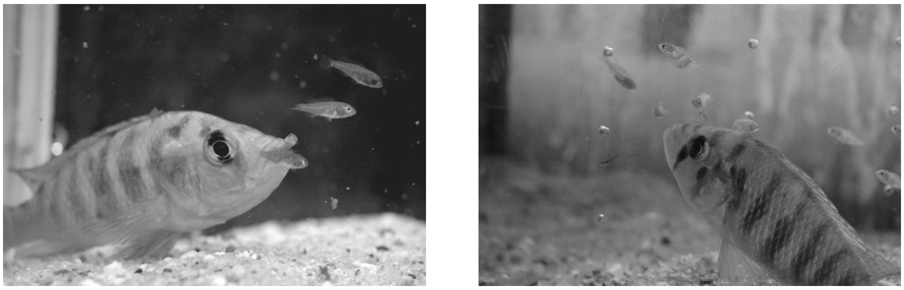

Figure 1. A *P.pundamilia* female providing brood care to newly hatched fry. Photos: J. van Heusden

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?

Chapter 2

Female mate choice behavior and sympatric speciation.

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Abstract

Many models have investigated how the process of speciation may occur in sympatry. In these models individuals are either asexual or mate choice is determined by very simple rules. Females, for example, may be assumed either to compare their phenotype to that of a potential mate, preferring to mate with similar males ("phenotype matching"), or to possess "preference genes" which determine which male phenotype they prefer. These rules often do not reflect the mate choice rules found in empirical studies. In this paper, we compare these two modes of female choice with various types of sexual imprinting. We examine the efficacy of different mate choice behavior in causing divergence in male traits 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). We 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 result of the model is similar to phenotype matching, where speciation can occur fairly easily. When females imprint on their fathers or imprint obliquely, speciation becomes considerably less likely. 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 suspect.

Key words: cultural evolution, female preference, phenotype matching, sexual imprinting, sympatric speciation.

Introduction

Using mathematical models to explore complex evolutionary phenomena always requires a number of simplifying assumptions. Many sympatric speciation models, for example, include a simple behavioral basis of assortative mating. In some models, mate choice is based upon phenotypic matching between the same trait in males and choosy females (Dieckmann & Doebeli 1999). In others, mate choice depends upon a match between an inherited female preference and a separate male trait (i.e. Kondrashov & Kondrashov 1999). Whether or not traits (or traits and preferences) match is simply a function of the difference between their phenotypic values. These simplified models can help to determine whether sympatric speciation is possible under a variety of circumstances.

We know, however, that assortative mating in nature is often not a simple matter of matching phenotypes. Learning, or sexual imprinting, often influences the mating choices of females. Some early work on the impact of learning on the divergence of populations had conflicting outcomes, and used assumptions now found to be unlikely or rare, such as male dominated mate choice and absolute preferences (Seiger 1967; O'Donald 1960; Kalmus & Smith 1966). A number of recent papers have stressed the importance of investigating how imprinting processes may have influenced evolution at various levels (Aoki et al. 2001; Laland 1994; Owens et al. 1999; ten Cate & Bateson 1988; Weary et al. 1993). Here, we will assess how assumptions about the ontogeny of female mating preferences influence models of sympatric speciation. The goal is not to explore every facet of female behavior, or to create a necessarily realistic model of sympatric speciation, but to broadly demonstrate how certain simple assumptions may influence conclusions drawn about sympatric speciation.

In our most basic model of female behavior, "phenotype matching", we assume that females prefer to mate with a male that shares their trait (we use this term as shorthand for "self-referent phenotype matching"). Although there is limited evidence for this exact mechanism (for review see Hauber & Sherman 2001), it could occur if females assess their own phenotype and use it as a basis for mate choice. Juvenile brown headed cowbirds, for example prefer to associate with adults that have the same feather colour and vocalisations as themselves, even though they have never seen a conspecific individual (Hauber et al. 2001). Likewise, females in the Australian frog (*Uperoleia rugosa*) prefer to mate with males that are about 70% (range 65 - 79 %) of their own body weight; correspondingly, clutches are only successful when the male is within 64 – 80 % of the females bodyweight (Robertson 1990). A similar mechanism of phenotype matching has also been invoked in the sympatric speciation of fish in Cameroonian volcanic lakes (Schliewen et al. 1994; Schliewen et al. 2001). In addition to self-reference, phenotype matching could also occur if females differentially aggregated with or encountered males with a similar phenotype to their own (dependent on the particulars of encounter probabilities). One final possible mechanism that would result in this mating pattern is a pleiotropic effect of genes on the mating trait and the mating preference. The general mechanism of phenotype matching forms the basis of assortative mate choice in the models of Kondrashov and Kondrashov (1999) and Dieckmann and Doebeli (1999). We examine several variants of this basic model.

The first three variants assess the effect of sexual imprinting. Females can 1) imprint on the phenotype of their mothers, 2) imprint on the phenotype of their fathers, or 3) imprint obliquely on members of the population at large. Although these three patterns differ in whom females learn from, they might entail somewhat similar social learning mechanisms. Evidence for sexual imprinting has been found in a wide variety of animals, mostly in birds and mammals (ten Cate & Vos 1999). Few studies of parental imprinting, however, have determined which parent the young imprint upon. Imprinting on the maternal phenotype has been shown in species where only females provide care for their offspring, such as in various geese and duck species (e.g. Kruijt et al. 1982). In a study on imprinting on novel traits, Witte et al (2000) also showed that both sexes of the Javanese Mannikin (*Lonchura Leucogastroides*) imprint on the maternal phenotype, rather than on the paternal phenotype. Less evidence exists that female offspring imprint on their fathers. Weisman et al. (1994) found that female zebra finches (*Taeniopygia guttata*) imprint on their fathers' beak color if the parents were not alike. Female zebra finches also were found to sexually imprint on novel traits of their father, but not their mother (Witte & Sawka 2003). However, another study also showed evidence for maternal imprinting by females in this species (Vos 1995). The scarcity of evidence for a paternal imprinting mechanism in female offspring probably reflects a dearth of studies investigating this phenomenon (Vos 1995). Oblique imprinting also appears somewhat uncommon, and has received little attention in classical imprinting studies. However, a recent study on wolf spiders (Hebets 2003) provides some evidence that this mechanism is found in nature. In this study sub-adult female wolf spiders exposed to a sexually active male with a certain phenotype later biased their adult mate choice towards this phenotype. The phenomenon of oblique imprinting also bears some resemblance to the processes of mate choice copying, in the sense that females obtain their preference from an unrelated individual in the population. Mate choice copying has been found in species including guppies (*Poecilia reticulata*) (e.g. Dugatkin & Godin 1992) and mollies (*Poecilia latipinna*) (Witte & Noltemeier 2002).

In the final variant, we consider a model where assortative mating is based on specific female preference alleles. Here females with one preference allele prefer a certain trait, while females with an alternative preference allele prefer another trait. In order to conclusively prove that female preferences work this way in a certain system, one would have to assess the genetic basis of preferences and/or rule out that preferences are based on imprinting or assessment of the female's own phenotype; few studies go to these lengths. Genetic variation in preferences that strongly suggests this type of genetic control has, however, been found in a variety of taxa, including Drosophila (e.g. Noor et al. 2001), swordtails (*Xiphophorus cortezi*) (Morris et al. 2003), and crickets in the genera Ephipigger and Laupala (e.g. Ritchie 2000; Shaw 2000).

We investigate the effect of preference inheritance on sympatric speciation in two ways. First, each mode of preference behavior is analyzed in a simple population genetic model. Secondly, we implemented each behavior in individual based simulation models based on those developed by Dieckman and Doebeli (Dieckmann & Doebeli 1999; Doebeli & Dieckmann 2000).

We find that these behavioral details of preference inheritance can have a profound effect on the chance of sympatric speciation. The 'phenotype matching' model is most favorable to speciation, under both modeling methods. The results of the variant where females imprint on their mothers are similar to those of phenotype matching. However, when females imprint on their fathers, conditions for sympatric speciation become more stringent. Furthermore, we find that populations with oblique imprinting are incapable of sympatric speciation. Finally, when female choice depends on preference genes the male trait may evolve easily, but the correlation between the trait and preference is not always very strong. We discuss the differences between the model variants and the implications for sympatric speciation.

The Models

In all of our models, we assume that males express a genetically transmitted trait that is used as a mating cue by females. The different inheritance mechanisms of mating preferences (whether learned or genetic) for this mating cue define the differences between the models. We examine the impact of these differences on the likelihood of speciation via two different approaches.

The first approach is to construct a very simple population genetic model that incorporates the female choice behavior. We are interested in the degree of facilitation of sympatric speciation by various forms of mate choice, and not in the various sources of selection and population dynamics that may produce and maintain two newly speciated populations in sympatry. Furthermore, recent work shows that sexual selection alone is unlikely to drive sympatric speciation under most conditions (Arnegard & Kondrashov 2004). We therefore simply assume that there is ecologically based negative frequency dependent selection on a male mating character that maintains the necessary genetic variation in a speciating population. Our analysis of the resulting models allows us to make some general conclusions about the influence of the different mate choice behaviors. The second approach that we use in the paper is to implement more complex individual-based simulation models of the evolution of reproductive isolation based on Dieckmann and Doebeli (1999), one of the more successful recent attempts to model sympatric speciation. In these models, individuals' mating traits are determined by a finite number of diploid loci, acting additively.

These two approaches examine the same preference inheritance mechanisms, however they ask slightly different questions about the speciation process. In the analytical approach, for example, we assess speciation by examining the relative stability of a polymorphism in the mating trait and by determining the degree of non-random mating in the population. In the simulation approach, on the other hand, we study speciation occurring as bifurcation of the population based on the mating trait.

Analysis of Population Genetic Models

The models describe evolution at a single trait locus by sexual selection in very large populations. We assume individuals are haploid; on the basis of simulations, we believe that this assumption does not qualitatively affect our conclusions regarding comparisons between the models. We also assume that all females mate once, and that generations are discrete and non-overlapping.

Females mate according to their preferences, based on our specific models of female behavior. We examine variation at the trait that acts as a mating cue, which is expressed in both sexes. The mating trait is controlled by locus T with alleles T1 and T2. We form three recursion equations for each model (details are presented in models 1-4 below): 1) the change from one generation to the next in the frequency of trait T1, 2) the change in the frequency with which females prefer T1, which we denote by Tx1, and 3) the change in D. D is a measure of disequilibrium between the locus T and the female preference for T. In other words, it indicates the level of association between preferences and traits. It is analogous to, and calculated in the same way as, the more familiar linkage disequilibrium between two genetic loci (used in model 5 below), or the "gene-culture disequilibrium" of Feldman & Cavalli-Sforza (1984).

As stated above, sexual selection alone is unlikely to drive sympatric speciation. These simple models are unable to result in a stable polymorphism for the trait with only divergent sexual selection as driving force. We therefore incorporate negative frequency dependent natural selection, *s*, to maintain genetic variation at this mating trait. This parameter selects against the mating trait, T1 or T2, that has a larger frequency than 1/2 in the total population. We will use the strength of s needed to maintain this polymorphism as one of the measures to compare the relative ease with which each assortative mating regime maintains two incipient species. Frequency dependent natural selection affects the frequencies of T1, Tx1 and D in models 1-4 after the formation of the zygotes, but before sexual reproduction, as follows:

$$t_1^* = t_1 \frac{1-2s(t_1-1/2)}{1-4s(t_1-1/2)^2} \qquad [1a]$$

$$t_{x1}^* = t_{x1} + D\frac{2s(1-2t_1)}{1-s(1-2t_1)^2} \qquad [1b]$$

$$D^* = D\frac{1-2s^2(t_1-1/2)^2}{(1-4s(t_1-1/2)^2)^2}$$
[1c]

Where t_1 is the frequency of T1 individuals before selection at time t and t_1^* is their frequency after selection; t_{x1} is the frequency at which trait T1 is preferred and t_{x1}^* is that frequency after selection; D* is the disequilibrium between t_1^* and t_{x1}^*.

Model 1: phenotype matching

This model, over the others in this study, bears most resemblance to several earlier models of speciation (Maynard Smith 1966; Udovic 1980; Kondrashov & Kondrashov 1999; Dieckmann & Doebeli 1999; Servedio 2000). Here a female's preference is determined by comparing her own expression of the trait T with the expression of that same trait in a potential mate. Thus T1 females prefer to mate with T1 males over T2 males by a factor $1+\alpha$ (and likewise, T2 females prefer to mate with T2 males over T1 males by the same amount). Here the preference specifically defines how much more likely a female would be to mate with one type of male over another type if she were to encounter one of each. Mating occurs assortatively, according to the described preferences above, resulting in the following recursion equations for the frequency of T1 and the preference for T1 at time t+1:

$$t_1(t+1) = t_1^* + \frac{\alpha t_1^* t_2^*\left(t_1^* - t_2^*\right)}{2\left(1+t_1^*\alpha\right)\left(1+t_2^*\alpha\right)}$$
[2a]

$$t_{x1}(t+1) = t_1(t+1)$$
[2b]

Table 1. Mating table for models 2-4: imprinting on mother, father and oblique imprinting models. Females are separated by trait and preference, where T12 is a female bearing trait T1 and preferring to mate with a male with trait T2. The matings are normalised for each female gene-preference combination, ensuring that each female mates once.

	Males	
Females	T1	T2
T11	$\frac{t_1^* t_{11}^*(1+\alpha)}{1+t_1^*\alpha}$	$\frac{t_2^* t_{11}^*}{1+t_1^*\alpha}$
T12	$\frac{t_1^* t_{12}^*}{1+t_2^*\alpha}$	$\frac{t_2^* t_{12}^*(1+\alpha)}{1+t_2^*\alpha}$
T21	$\frac{t_1^* t_{21}^*(1+\alpha)}{1+t_1^*\alpha}$	$\frac{t_2^* t_{21}^*}{1+t_1^*\alpha}$
T22	$\frac{t_1^* t_{22}^*}{1+t_2^*\alpha}$	$\frac{t_2^* t_{22}^*(1+\alpha)}{1+t_2^*\alpha}$

The recursion for the frequency of the preference for T1, t_{x1} is the same as the recursion for t_1, since each individual has a preference for its own trait by definition. Disequilibria between the preference and the trait are calculated in a way analogous to genetic disequilibrium (e.g., D = $t_{11}t_{22} - t_{12}t_{21}$ where t_{21} is the frequency of individuals with trait T2 and preference Tx1). The value of D for phenotype matching will be t_1t_2. This is a trivial consequence of the fact that under phenotype matching t_{12} and t_{21} are 0 by definition; the relevance of D will become apparent in comparison with the other models.

The following three models, where females imprint on their mother, father, or obliquely on males in the parental generation, are modifications of the phenotype matching model. In all three of these models, each phenogenotype of preference and trait is affected through selection on the trait alone. The only difference between the models is the subject of sexual imprinting for the females.

Model 2: maternal imprinting

In this model, a female's preference for either T1 or T2 is determined by the phenotype of her mother. Females that had a mother bearing the T1 trait will prefer to mate with a T1 male over a T2 male by a factor 1+*α*, while females with a mother bearing T2 would prefer to mate with a T2 male by the same factor. Table 1 shows how the frequencies of the crosses are determined. In the appendix, section 1, we show which cells of the table contribute to each recursion equation. The resulting recursion equations are:

$$t_1(t+1) = t_1^* + \frac{1}{2}F \quad , \tag*{[3]}$$

where

$$F = \frac{\alpha t_1^* t_2^*}{(1+t_1^*\alpha)(1+t_2^*\alpha)}\left(t_{x1}^* - t_{x2}^* + \alpha(t_{x1}^* - t_1^*)\right) \quad . \tag*{[3a]}$$

$$t_{x1}(t+1) = t_1^* \tag*{[4]}$$

$$D(t+1) = \frac{t_2^* t_1^*(1+\alpha+2\alpha D^* + \alpha^2(D^* + t_1^* - t_1^{*2}))}{2(1+t_1^*\alpha)(1+t_2^*\alpha)} \tag*{[5]}$$

Here the number of females preferring T1 is simply the frequency of mothers with T1.

Model 3 Paternal Imprinting

Female preferences for either T1 or T2 are determined by the phenotype of the father, where a female with a T1 father will prefer to mate with a T1 male over a T2 male by a factor 1+a. This model is otherwise the same as model 2. The change in the ontogeny of the female preference results in different recursion equations for t_{x1} and D, while the expression for t_1(t+1) remains the same as in model 2, equation [3] (see appendix section 1).

$$t_{x1}(t+1) = t_1^* + F \qquad [6]$$

$$D(t+1) = \frac{1}{2}\left(t_1^* \left(\frac{(1+\alpha)(t_1^* t_{x1}^* + D^* + t_{x1})}{1+\alpha t_1^*} + \frac{t_{x2}^*(1+t_1^*) - D^*}{1+\alpha t_2^*} - 2t_1^* - 3F \right) - F^2 \right) \qquad [7]$$

In equations [6] and [7], F is the same expression given in equation [3a]. This difference between model 2 and model 3 stems from the fact that fathers, unlike mothers, have unequal mating success, so t_{x1}(t+1) attains the more complicated form seen here.

Model 4: oblique Imprinting

Here, females 'imprint' obliquely on the phenotype of the males of the previous generation. In other words, the chance that a female will have a preference for trait T1 is proportional to the frequency of that trait in her parents' generation. There are two plausible biological scenarios that might cause this: either females choose (or encounter) a random male from their parents' generation to imprint upon; or females simply choose whether they prefer T1 or T2 based on how common these phenotypes were in their parents' generation.

The recursion equations for t_1 and t_{x1} in this model are the same as in model 2 (see appendix section 1). The recursion for D is different, however. Because the chance that a female will imprint on T1 has no relation to the chance that she also bears this trait, D is always 0.

Model 5: genetic preference for mating trait

In this model, female preferences are based on a separate genetic locus P (similar to Kirkpatrick 1982). This preference locus has two alleles, P1 and P2, which correspond to a preference for either of the two male traits. Unlike Kirkpatrick (1982), and the models above, we analyze the simple situation where natural selection on males is absent. Negative frequency dependence is not necessary to maintain a polymorphism, as in the other models, because here there is a line of stable polymorphic equilibria even without selection. The strength of preference of P1 females for T1 males over T2 males is 1+a, and vice versa, where the strength determines the likelihood of mating with the preferred male when one male of each type is encountered.

The frequencies of mating are determined following the mating table (table 2), which is a modification of the mating table in Kirkpatrick (1982, table 1). When individuals have mated, the genotype of their offspring is affected by the

	Males			
Females	T1P1	T1P2	T2P1	T2P2
T1P1	$\frac{x_1^2(1+\alpha)}{1+t_1^*\alpha}$	$\frac{x_1x_2(1+\alpha)}{1+t_1^*\alpha}$	$\frac{x_1x_3}{1+t_1^*\alpha}$	$\frac{x_1x_4}{1+t_1^*\alpha}$
T1P2	$\frac{x_2x_1}{1+t_2^*\alpha}$	$\frac{x_2^2}{1+t_2^*\alpha}$	$\frac{x_2x_3(1+\alpha)}{1+t_2^*\alpha}$	$\frac{x_2x_4(1+\alpha)}{1+t_2^*\alpha}$
T2P1	$\frac{x_3x_1(1+\alpha)}{1+t_1^*\alpha}$	$\frac{x_3x_2(1+\alpha)}{1+t_1^*\alpha}$	$\frac{x_3^2}{1+t_1^*\alpha}$	$\frac{x_3x_4}{1+t_1^*\alpha}$
T2P2	$\frac{x_4x_1}{1+t_2^*\alpha}$	$\frac{x_4x_2}{1+t_2^*\alpha}$	$\frac{x_4x_3(1+\alpha)}{1+t_2^*\alpha}$	$\frac{x_4^2(1+\alpha)}{1+t_2^*\alpha}$

Table 2. Mating table for model 5: preference genes model.
The frequency of T1P1 after mutation is noted by x_1, T1P2 by x_2, T2P1 by x_3 and T2P2 by x_4. Matings are normalised so that each female mates once.

recombination rate, r, between the preference and the trait loci.

Modifying the analysis in Kirkpatrick (1982) to determine the recursion equations for t_1, p_1 and D, where D in this case is the traditional gene-linkage disequilibrium, and p_1 is the frequency of P1 individuals at time t, we find that

$$t_{1(t+1)} = t_1^* + \frac{1}{2}t_1^*(H-1) \qquad [8]$$

$$p_{1(t+1)} = (t_{1(t+1)} - t_1^*)(\frac{D}{t_1^*(1-t_1^*)}) \qquad [9]$$

$$D_{(t+1)} = D + \frac{1}{4}[G+H+GH-3] - \frac{1}{4}S[1+Gt_1^*+Ht_2^*] , \qquad [10]$$

where

$$G = \frac{(\alpha+1)p_1^*}{1+t_1^*\alpha} + \frac{p_2^*}{1+t_2^*\alpha} ,$$

$$H = \frac{p_1^*}{1+t_1^*\alpha} + \frac{(\alpha+1)p_2^*}{1+t_2^*\alpha} , \text{ and}$$

$$S=r\left[\left(\frac{2-\alpha}{1+t_1^*\alpha}-\frac{\alpha}{1+t_2^*\alpha}\right)\left(t_2^*t_1^*p_2^*p_1^*+D^2\right)+D\left(\frac{1}{1+t_1^*\alpha}+\frac{1}{1+t_2^*\alpha}\right)\left[\left(t_1^*p_1^*+t_2^*p_2^*\right)+\left(t_1^*p_2^*+t_2^*p_1^*\right)(\alpha+1)\right]\right]$$

Equilibria and Stability

We solved these models for their equilibria, on which we performed a local stability analysis. We were then able to compare the stability of the models by comparing the strength of the frequency dependent selection coefficient, *s*, that switches a polymorphic equilibrium, found in all models, from stable to unstable (bifurcation point; see appendix sections 2 and 3). This produces a curve (the "*s*-curve") for each model, expressing the switch point for *s* in terms of *α*. We used the level of disequilibrium, D, at these stable polymorphic equilibria as another indication of progress towards sympatric speciation. Models 1-4 all have several equilibria (see appendix), of which all models have three biologically relevant equilibria in common; two where one of the two trait alleles is extinct, and one where the frequencies are $t_1=t_2=½$. However, the equilibrium level of disequilibrium, $\hat{D}$, at this polymorphic equilibrium differs between all models (table 3).

Model 5 behaves somewhat differently than the other models that we have introduced. Instead of one internal equilibrium, there is a line of stable equilibrium which is expressed by the following equations:

When: $\frac{1}{2+\alpha} < \hat{p}_1 > \frac{1+\alpha}{2+\alpha}$, $\hat{t}_1 = \hat{p}_1\frac{2+\alpha}{\alpha} - \frac{1}{\alpha}$

Otherwise: $\hat{t}_1 = 0,\ \hat{p}_1 \leq \frac{1}{2+\alpha}$ or $\hat{t}_1 = 1,\ \hat{p}_1 \geq \frac{1+\alpha}{2+\alpha}$

The minimum frequency dependent selection, *s*, required for the polymorphic equilibria to be stable depends differently on the strength of the preference, α, in all learning models. Increasing *s* makes the polymorphic equilibria more likely to be locally stable and the edge equilibria less likely to be locally stable. The maximum frequency dependent selection value, *s*, at which the edge equilibria are stable, however, is the same among these models. The equations of these relations are shown in table 3, and the corresponding curves are plotted in figure 1.

Because the *s*-curves for the edge equilibria are not identical to any of the *s*-curves for the polymorphic equilibra in the models, there is an area in each model where there are 3 stable equilibria. Numerical iterations have shown however, that in this area the range of t_1 for which the edge equilibria are locally stable is marginal and that the separatrix determining movement towards an edge equilibrium is close to the edge. This indicates that once the

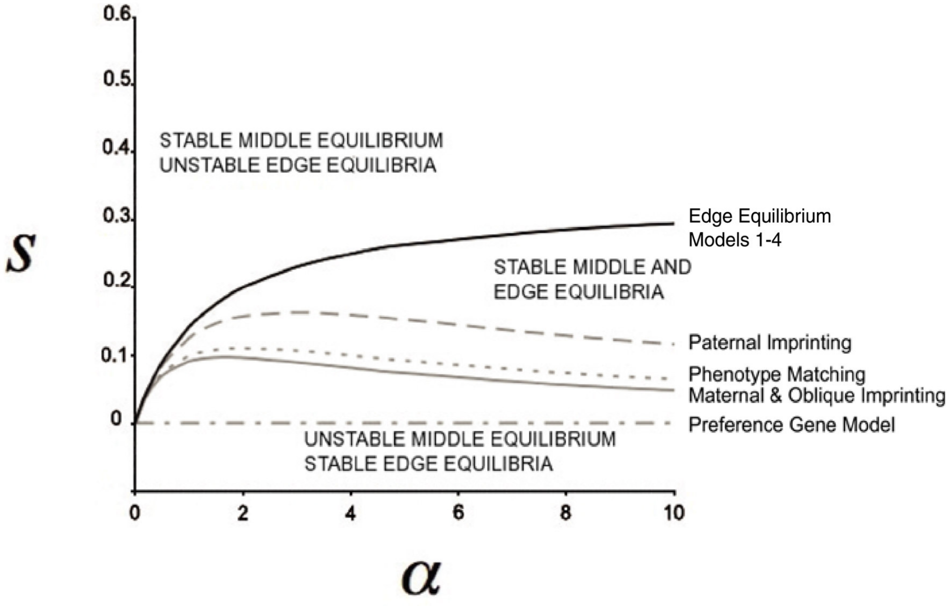

Figure 1. The dependence of *s*, the value of frequency dependent selection needed for each equilibrium point to be stable, on *α*, the strength of the preference for a certain trait. The middle equilibrium is the polymorphic equilibrium. All values of *s* above the gray line for each model will give a locally stable polymorphic equilibrium, while all values of *s* below the gray line will make the polymorphic equilibrium locally unstable. Models 1-4 have the same curve for the edge equilibria, which also shows the dependence of *s* on *α*, where here the curve shown is the maximum frequency dependent selection value for which the edge equilibria will be locally stable. The preference gene model has a line of stable equilibria without frequency dependent selection, and thus does not depend on *s*.

conditions for a stable polymorphic equilibrium are met, the models move towards the polymorphic equilibrium over most of the parameter space.

We concentrate our comparisons between the models on the stability of the polymorphic equilibrium because this shows the ability of each model to maintain two species in sympatry. The ability to maintain a polymorphism is both a prerequisite for speciation and potentially an important determinant of whether two incipient species can continue to co-occur. Stability of the polymorphic equilibrium is also particularly important in our model because of the relatively small area of the parameter space in which the population will evolve towards the edge equilibria. We therefore use the range of parameters for which the polymorphic equilibrium is stable as a measure of the ease of the generation and maintenance of speciation in sympatry.

Table 3. Summary of results of population genetics models.

The minimum s is the lowest value of the frequency dependent selection coefficient, s, required to maintain a polymorphism for the mating trait. See appendix for derivation of these equations. 'D' at equilibrium results in equal equations for maternal and paternal imprinting models. Outside this equilibrium point, however, the disequilibrium in the paternal imprinting model is lower than in the maternal imprinting model.

Model type	1 Phenotype matching	2 Maternal imprinting	3 Paternal imprinting	4 Oblique imprinting	5 Preference gene
Minimum s for a locally stable polymorphic equilibrium	$s = \frac{\alpha}{4+5\alpha+\alpha^2}$	$s = \frac{2(4\alpha+\alpha^2)}{32+52\alpha+22\alpha^2+3\alpha^3}$	$s = \frac{2(4\alpha+\alpha^2)}{32+36\alpha+10\alpha^2+\alpha^3}$	$s = \frac{\alpha}{4+5\alpha+\alpha^2}$	0
Maximum s for a locally stable edge equilibrium	$s = \frac{\alpha}{4+3\alpha}$	$s = \frac{\alpha}{4+3\alpha}$	$s = \frac{\alpha}{4+3\alpha}$	$s = \frac{\alpha}{4+3\alpha}$	0
$\hat{D}$ at polymorphic equilibrium	$\hat{D} = 1/4$	$\hat{D} = \frac{2+\alpha}{16+4\alpha}$	$\hat{D} = \frac{2+\alpha}{16+4\alpha}$	$\hat{D} = 0$	$\hat{D} > 0$, but lower than in models 1,2&3 (see appendix for expression)

Results of the population genetic models

As mentioned above, we considered two parameters of the models important in the assessment of the evolution of reproductive isolation in sympatry. The first of these is the tendency to maintain a polymorphism in the mating trait, which we assessed by measuring the strength of negative frequency dependent selection needed for local stability of the polymorphism. This selection prevents the positive frequency dependent selection created by mating from causing the population to converge on one mating trait. The second measure is the strength of disequilibrium between trait and preference. This is a critical factor, because without this association there would be one polymorphic population instead of two, reproductively isolated, emerging subpopulations or species. It is not immediately apparent how these two parameters trade off to determine whether speciation can be considered to be occurring, although we discuss the significance of these measures below.

In table 3 and figures 1 and 2 we have summarized the analytical evaluation of our models in terms of the trait-preference disequilibrium and the minimum amount of *s* required to produce an internal equilibrium.

The preference gene model (model 5) has the easiest conditions for a stable internal equilibrium, since in this model there exists a stable line of equilibrium between the loss and fixation of the allele T1. This stable internal equilibrium exists even without negative frequency dependent selection. This results from the fact that all females have equal mating success, regardless of their preference allele. Preference alleles can therefore be stable at any frequency, and a corresponding stable frequency of the trait allele will result. However, disequilibrium in this model is relatively weak, since an individual's preference is not directly related to its own trait phenotype. In comparison, the phenotype-matching model (model 1) obviously has a very high disequilibrium value, but on the other hand, requires more stringent conditions for stability.

Both the maternal and paternal imprinting models (models 2 and 3, respectively) have slightly lower disequilibrium values than does phenotype matching. As long as there is any interbreeding between mating types, either of an individual's parents might not possess the same mating trait as the individual itself. The disequilibrium in these models is still considerably higher than that in the preference gene model. The stability of the internal equilibrium in the maternal imprinting model is similar to that of the phenotype matching model, however, it requires slightly lower negative frequency dependent selection to be stable. Paternal imprinting on the other hand, seems to cause greater positive frequency dependence, and hence requires much stronger

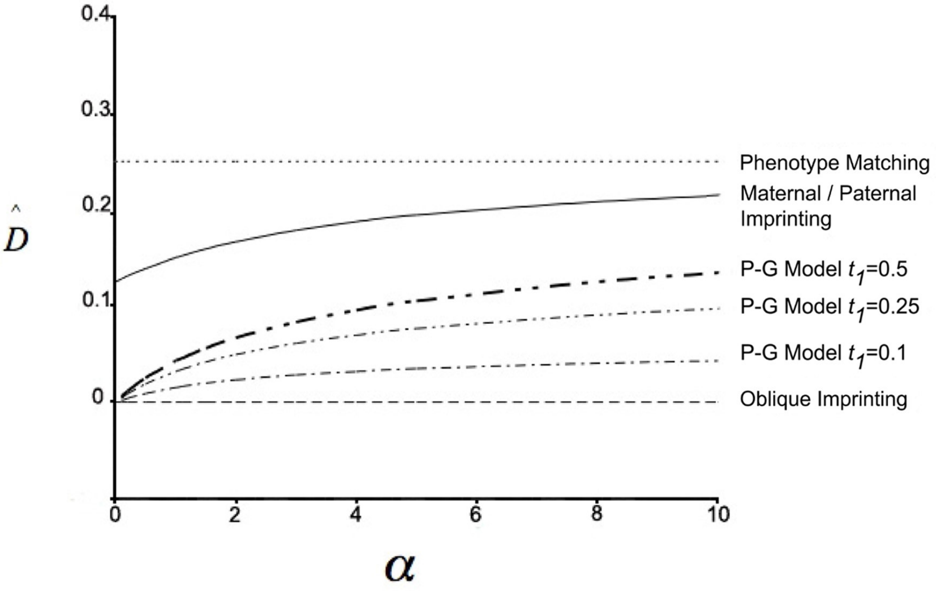

Figure 2. The level of preference trait disequilibrium at the polymorphic equilibrium, t_1=1/2. The phenotype matching model is at maximal disequilibrium. For the preference gene model three examples of disequilibrium along the equilbrium line are shown and their corresponding values of p_1 are given in the appendix, figure A-1.

negative frequency dependence for stability of its internal equilibrium (see figure 1).

The conditions for speciation are most stringent with oblique imprinting. Despite the relatively lax conditions for maintaining a stable internal equilibrium, in this model, disequilibrium cannot be established. We therefore consider oblique imprinting incapable of driving speciation.

Individual Based Simulations

The results of the analytical models above have several limitations. First, it is not obvious how the two factors of gene-preference disequilibrium and the stability of the polymorphic equilibria will trade-off to determine the ease of speciation. Second, the models are very simplified. For example, phenotypic differences between two species, including sexual signals, are often controlled by multiple genetic differences. Recent models with this level of complexity have used individually-based computer simulations (Dieckmann and Doebeli 1999, 2000), following earlier individual-based simulations of speciation (Kulagina & Lyapunov, 1966, Menshutkin, 1977, Kondrashov, 1980, 1986). We implemented an individual-based model of this type, based on a simplified version of the Dieckmann and Doebeli (1999, 2000) model. Individuals possessed 5 unlinked diploid loci (with alleles T and t). An individual’s phenotype, x, is the sum of all the T genes it possesses. In other words, genetic interactions are strictly additive and the effects of all loci on the phenotype are equal. The mutation rate of these genes was 0.00001 per locus. All females mated once per “year”. Females possessed a preference for one type of phenotype, y. This type of phenotype was either their own phenotype (phenotype matching, corresponding to model 1 above); the phenotypic output of their preference loci (corresponding to model 5 above); either their mother or father’s phenotype (corresponding to models 2 and 3 above respectively); or the phenotype of a randomly selected male from the population (oblique imprinting, corresponding to model 4 above). In the case, similar to model 5 above, where females possessed separate preference loci, individuals also possessed 5 further unlinked, additive loci to determine the preference phenotype. The chance that a female would mate with a given male depended on the difference between her preferred phenotype and his phenotype. As this difference increased, the probability of mating declined according to a Gaussian distribution (Dieckman and Doebeli 1999), with variance a (a measure similar to a in the models described above). The preference, p, of a female with preferred phenotype yf for a male with phenotype xm was therefore:

$$p = e^{\frac{-(x_m + y_f)^2}{a^2}}$$

The preference of a female for a given male phenotype was normalized over the distribution of male phenotypes to ensure that all females mated, even if their preference was very different from the available male phenotypes (this has a conservative effect on speciation). In our models, we investigated different parameter values of a, but within a simulation, female preferences did not evolve. This was different from Dieckman & Doebeli's simulations, where female preferences evolved, but to a maximum of a=0.05, a value that was fixed in all the results they reported.

We modified Dieckman & Doebeli's model by removing ecological variation. The model incorporates overlapping generations, with all individuals facing the same risk of dying in each "year", irrespective of their phenotype. The mortality rate was determined by a simple frequency-dependent relation that ensured a relatively constant population size: rN/K . Here r is the birth rate, N is the population size, and K is a parameter of the carrying capacity of the population. r was set to 1, and K to 15000 by default, causing an equilibrium population size of around 5000 individuals.

The evolutionary trajectory of such a system can be followed. In the case of speciation, it is necessary that polymorphisms are maintained and that disequilibrium builds up between these loci such that some individuals have a preponderance of T alleles, while others have primarily t alleles, causing a bifurcation of phenotypes. From a phenotypic perspective, this results in reproductive isolation between the two groups of individuals. In this model, even without ecological competition, assortative mating can still cause bifurcation of a population into two subpopulations, as predicted by Shpak & Kondrashov (1999).

In the simulations we measured whether the population equilibrated in one of three states: fixation of the population on one genotype; or on a range of genotypes without reproductive isolation; or finally, fixation of the population on two (or more) reproductively isolated genotypes. Only the latter case counts as speciation.

At the beginning of each run of the simulation, each individual's genotype was determined at random (for each gene there was a 50% probability of acquiring the T allele). Female preferences were set to be the same as their own phenotype in the cases of imprinting as well as assortative mating; in the case of preference genes, they were allocated the same values as the signal genes (these assumptions were not critical since rapid association of trait and preferences developed in the models anyway). Simulations ran for a period of 1000 "years" (equivalent to approximately 300 generations). To establish the threshold value, we carried out 10 repetitions of each parameter setting.

Our simulations can be found online at http://website.leidenuniv.nl/~lachlanrf/Simulation.html.

Simulation Results

Whether speciation occurred depended on several variables in the model: larger population sizes and higher mutation rates increased the genetic diversity in the population and tended to facilitate speciation. Of more interest was the effect of the preference variance (a): how quickly a female's preference dropped off as a male's phenotype became more unlike her ideal phenotype. At high levels of this parameter, when individuals tended not to have a strong preference for their preferred phenotype, speciation was prevented since too much recombination between individuals with different phenotypes occurred. We therefore measured the maximum level of a that resulted in speciation, and used this as an assessment of how easily a given set of parameters led to speciation (a higher a-max represents more permissive conditions for speciation).

Speciation occurred under all modes of female preference behavior we examined, except for oblique transmission. In this latter case, no linkage can develop between preference and mating trait, as discussed in the population genetics section above. In the remaining four models of mate choice, however, bifurcation occurred under different parameter ranges and to different degrees. Speciation occurred particularly easily with phenotype matching (a-max=0.15). From an initial central range of phenotypes, the population evolved so that there were two sub-populations that existed at

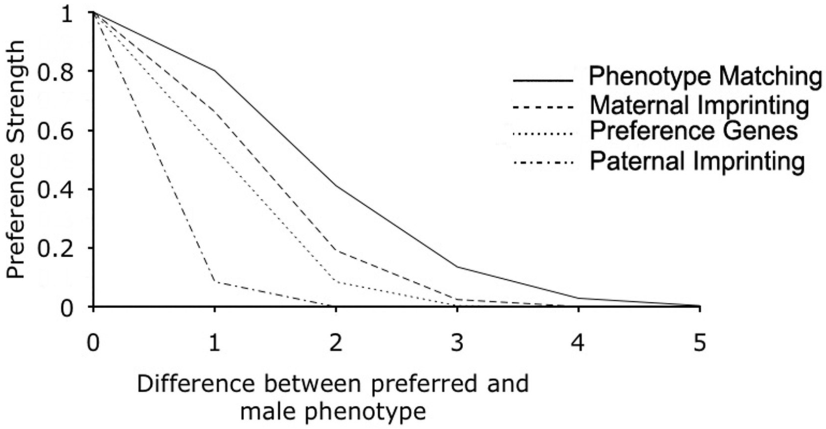

Figure 3. Shows the female preference functions at the speciation thresholds (amax) in the individual-based model. The more effective a type of female preference is at causing speciation, the weaker the female preference strength has to be to cause speciation. The figure shows the distribution of female preferences at the threshold values of a-max required to allow speciation. The y-axis values represent the strength of preference a female has for a given phenotype, relative to her preference for the most-preferred phenotype. For example if a female has to choose between two males, one with her most-preferred phenotype, and one with a preference score of 0.5, her probability of choosing the most-preferred phenotype is (1+0.5)/2=0.75. The x-axis shows the phenotype value of a male, in terms of how different to the most-preferred phenotype of the female.

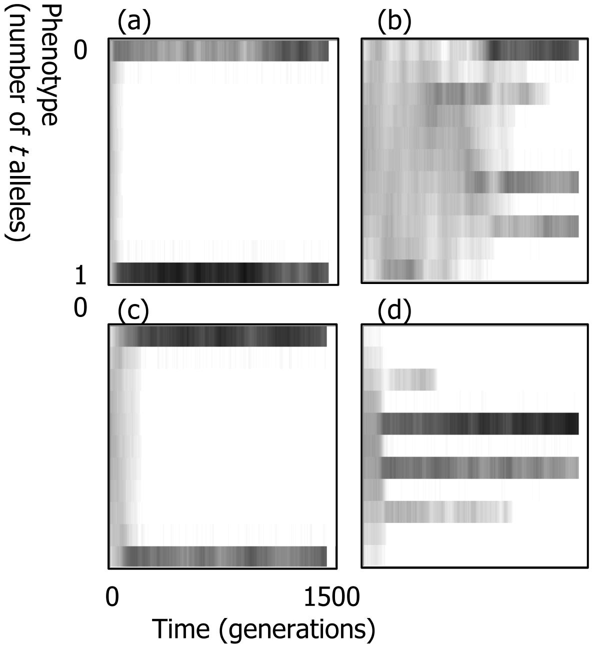

Figure 4. Representative runs of the simulation under the four mating modes (a: phenotype matching; b: preference loci; c: maternal imprinting; d: paternal imprinting). The x-axis represents time (the simulation proceeded for 1500 years); while the y-axis represents the value of the phenotype. The gray-scale represents how many individuals had that phenotype at that time (black = all individuals had that phenotype, white = no individuals had that phenotype). Under phenotype matching and maternal imprinting, bifurcation creates two subpopulations at extreme phenotype values, while different patterns are found with preference genes and paternal imprinting.

either extreme of the distribution, with all loci fixed for T in one sub-population and t in the other. When females imprinted on their mother there was a similar pattern of bifurcation, although the value of a-max, 0.11, was somewhat lower than for phenotype matching, translating into more stringent conditions for bifurcation (figure 3). When females imprinted on their fathers, however, there was a different pattern of bifurcation (figure 4), and a-max=0.045.

Rather than evolving to the extremes of the phenotypic space, when bifurcation occurred, populations evolved such that the two phenotypes were somewhat similar. In this case, there nevertheless tended to often be two genotypes fixed in the population, and each locus tended to lose variation. Finally, when females relied on preference loci to determine their mate choices, another pattern of bifurcation was observed (figure 4). Bifurcation did not lead to two subpopulations that were equally distant from the median phenotype; instead, the phenotypes seemed to be determined more or less at random. In this case too, a-max was considerably more stringent than for phenotype matching at 0.09.

Figure 3 shows how these values of a-max affect each model. With phenotype matching speciation occurred even if females were unable to clearly distinguish their most preferred phenotype from its neighbors in phenotype space. With maternal imprinting and preference loci the conditions for speciation are somewhat more restrictive. Under paternal imprinting, the conditions for speciation require that females nearly always mate with males whose phenotype matches their preference exactly. Finally, with oblique imprinting no speciation could occur at all.

Discussion

The main conclusion that can be drawn from our models is that the sympatric divergence of mating traits, determining the development of prezygotic isolation and hence the process of sympatric speciation, can be very sensitive to the behavioral basis of mate choice preference. The five different manners in which females acquired a mating preference all differed in how easily they maintained a polymorphism in the mating trait, in the linkage disequilibrium between preference and trait, and in how likely they were to bifurcate in a multilocus model. All three of these measures are likely to be important indicators of the potential to speciate.

Phenotype matching was our reference behavior, since this is the form of mate choice that most previous models incorporate (Maynard Smith 1966; Udovic 1980; Kondrashov & Kondrashov 1999; Dieckmann & Doebeli 1999; Servedio 2000). It is not clear how common this mechanism is in nature. It may be difficult, for example, for individuals of many species to assess their own color patterns or other mating cues. However, a review of the evidence for self-referent phenotype matching suggests that it may be a more common phenomenon than previously thought (Hauber & Sherman 2001). In the context of individual based simulations, this model facilitated ‘speciation’ the most, leading to bifurcation in the widest range of parameter values (see figures 3 and 4). By definition, the trait preference disequilibrium is always maximal in this model.

Maternal imprinting provided the closest approximation to phenotype matching. With phenotype matching, there is obviously a perfect match between a female’s preference and her own trait, while with maternal imprinting, the disequilibrium must be diluted to some extent. Therefore, the trait preference disequilibrium was slightly lower in the maternal imprinting model. Interestingly, however, the stability of the polymorphism was achieved with less help of negative frequency dependent natural selection (figure 1) in the maternal imprinting model than in the phenotype matching model. An explanation for this counterintuitive result lies in the difference between the two models in how the frequency of the female preference is determined. In the phenotype matching model, this is equal to the frequency of each trait in the current generation. In the maternal imprinting model, the frequency of the preference for a trait is equal to the frequency of that trait in the previous (parental) generation. This results in a time lag in the evolution of the preference distribution in the maternal imprinting model over that in the phenotype matching model. This in turn leads to an increase in the parameter range for stability of the polymorphic equilibrium, because the time lagging preference slows any movement of the population away from the equilibrium frequency.

In the individual based simulations, maternal imprinting also behaved most similarly to phenotype matching. Maternal imprinting is known to be present in birds (Kruijt et al. 1982; ten Cate & Vos 1999; Witte et al. 2000) and also occurs in a number of mammals (Kendrick et al. 1998), and is, to our knowledge, more prevalent than phenotype matching mechanisms. Its similarity to phenotype matching implies that the results of many previous phenotype matching models (e.g. Kondrashov & Kondrashov 1999; Dieckmann & Doebeli 1999) may apply to species that demonstrate maternal imprinting as well.

One of the principal results of this paper is that it matters greatly whom females imprint upon. In contrast to maternal imprinting, polymorphisms were less easily maintained with paternal imprinting. Correspondingly, bifurcation did not occur with paternal imprinting except under relatively extreme conditions in the individual based simulation. The difference between paternal and maternal imprinting in our models stems from the fact that all females had equal reproductive success, while males' reproductive success varied according to females' preferences. This meant that, with paternal imprinting, only successful males were imprinted upon, which increased the number of females preferring that type of male. This generates greater positive frequency dependence compared to the other models, which makes it difficult to maintain polymorphisms.

Finally, imprinting on unrelated males, unsurprisingly, made it impossible to establish prezygotic isolation; under these conditions no disequilibrium between trait and preference can ever develop. In summary, while phenotype matching is unlikely in reality, imprinting may cause similar evolutionary patterns, but only when females imprint on their own mothers.

Our final comparison was with a model incorporating preference genes. In this case, polymorphisms are maintained very easily indeed, because preferences are selectively neutral as long as all females have equal reproductive success (see Kirkpatrick 1982). On the other hand, recombination between the preference loci and the trait loci keeps the level of trait preference linkage disequilibrium in this model substantially lower than the trait - learned preference disequilibria in the sexual imprinting and phenotype matching models. It is unclear how to interpret the combination of these two factors in influencing the probability of speciation. We suggest, from the results of the individual based simulations, that speciation is considerably less likely with preference genes.

The ability of a system to maintain a polymorphism over time is a requirement for the formation of two distinct and isolated populations. The relative ability of a model to maintain a polymorphism is often seen in speciation models as the most important factor in influencing sympatric speciation. By contrast, trait-preference disequilibrium is seen as a less critical factor. Any disequilibrium at all that is measured in the analysis of such an equilibrium is interpreted as progress towards isolation of two incipient species, since it is assumed that further evolution of mate preferences will cause the disequilibrium to increase. In our deterministic models, we considered both the stability and the disequilibrium measures, and in comparing with the individual based models, it is clear that the level of disequilibrium does play a critical role. Even though there is significant disequilibrium in the preference gene model, it is less than in the phenotype matching model, and this translated to speciation, measured as bifurcation, occurring more readily with phenotype matching in the individual based models (figures 3 and 4).

The assumption that only females are choosy ensures that all females, unlike males, have equal reproductive success regardless of their phenotype or choice of mate. This in turn is important to the difference between the maternal and paternal imprinting models, as discussed above. In nature, females with an unusual preference may waste time, or may not even mate at all if they cannot find a suitable mate. A cost of having an unusual preference would make it less likely for all females to have equal reproductive success. This would reduce the difference in results from the maternal and paternal imprinting models. Males on the other hand, are not exerting a choice at all in our models. Mutual mate choice is another factor that could change the equal reproductive success of females, because a female with an unusual phenotype would have a reduced chance of finding a suitable mate. This last factor would affect the predictions for establishing a polymorphism, if we were to study how a new trait invading a population would fare. However, in our analysis we focus on the stability of an existing polymorphism, hence largely ignoring the dynamics associated with trait variation establishment.

Another aspect of speciation that was largely ignored in this paper is the evolution of the strength of mating preferences. This would probably affect our more detailed conclusions. A partially reproductively isolated population could become more isolated either by continuing divergence of the mating traits, or alternatively, by female preferences becoming more acute. In the case of the preference gene model (model 5 above), the mating traits did not always continue to diverge in the individual based simulations, because of the low trait-preference disequilibrium. This disequilibrium would have increased if the female preferences had been allowed to become stronger over time.

The mechanisms by which the females obtained their preferences were also not allowed to evolve in this study. We assumed that the evolution of these mechanisms is constrained. The pattern of occurrence of sexual imprinting in the phylogeny of birds suggests that imprinting is widespread, but the role it plays in the formation of sexual preferences varies (Immelmann 1975; ten Cate & Vos 1999). This may be an indication that the function of sexual imprinting can evolve, but the mechanism itself does not easily disappear. Furthermore, a few models show that learning of sexual preferences can indeed evolve (Servedio & Kirkpatrick 1996; Todd & Miller 1993), even without direct fitness benefit (Servedio & Kirkpatrick 1996).

Well-known examples of species that may have formed in sympatry are the cases of the haplochromine cichlids from the East African lakes. A long standing debate on the origin of the species richness in these lakes has recently focussed on sympatric speciation with sexual selection as a major driving force. Numerous sympatric species within a trophic group show remarkable radiation in color patterns, and there is growing evidence that many of these are the result of sympatric speciation (Seehausen et al. 1999; Allender et al. 2003; Albertson et al. 1999; Salzburger & Meyer 2004). Within these trophic groups often little ecological differentiation is found (Seehausen & Bouton 1997; Danley & Kocher 2001). Several experiments indicate the presence of disruptive sexual selection (Maan et al. 2004; Knight & Turner 2004; Seehausen & van Alphen 1998). For these cichlids it is largely unknown how their mate choice preferences are formed, however where tested their mate preference was found to be assortative.

In our analysis, we presume there is ample variation in mating traits by concentrating on the stability of a polymorphism. Radiation by hybridization may be one scenario that would provide this variation in traits. In a recent review, it was argued that hybridization could potentially even fuel adaptive radiation (Seehausen 2004). Hybridization of two species would create sudden (in only a few generations), broad variation in male traits in the new hybrid population. This broad variation may be a prerequisite for sympatric speciation, according to theory (e.g. Kondrashov & Kondrashov 1999; Dieckmann & Doebeli 1999; Higashi et al. 1999). The cichlid flock of Lake Victoria may have originated from a hybrid swarm that colonized the lake after a period of drought (Seehausen et al. 2003); the scenario of speciation by hybrid adaptive radiation has also been suggested for cichlid species from Lake Malawi (Smith et al. 2003). These aspects render our model potentially applicable to cichlid speciation.

Guppies are another example of species with a high degree of polymorphism in male color patterns. This polymorphism in nuptial coloration appears both between different populations and within certain populations, but does not appear to result in speciation. It has been shown that females' mate choice preferences in guppies are influenced by mate choice copying (Dugatkin & Godin 1992), although they appear to have a genetic basis for preference ranges as well (Brooks 2002). Mate choice copying has a crucial property in common with our model of oblique imprinting, in that it involves preference learning from an unrelated individual. This hinders the build up of any trait-preference disequilibrium, making divergence based on sexual selection nearly impossible as long as there is any degree of gene flow between populations.

In conclusion, we show that assumptions concerning the way females form their mate choice preferences can have potentially profound influences on the predictions for speciation. Although phenotype matching mechanisms have been used very often in general models of speciation, they are probably not common mechanisms in nature. Sexual imprinting, on the other hand, is quite widespread. We find that the type of sexual imprinting can affect speciation. While speciation occurs almost as readily in our maternal imprinting model as with phenotype matching, we find it much less likely to obtain with paternal imprinting. We also conclude that speciation cannot be driven by oblique imprinting. Finally, we find that the model with a genetic basis for mate choice preference behaved very differently from our other models, and may not always facilitate speciation. In nature, many species may have a mixture of these mechanisms to form their preferences, such as genetic predispositions to imprint on certain traits more than on others (Bolhuis 1996; ten Cate 1989). This study shows that the knowledge about how species form their preferences will improve our understanding of speciation processes.

Acknowledgments

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Appendix

1) Recursion equations models 2-4

The recursion equations in models 2-4 are determined using table 1. Which cells of the table make up each equation is explained below.
Individuals carrying trait T1 that have a preference for T2 individuals, are denoted by T12. Likewise, individuals with trait T2 and a preference for trait T1, are denoted by T21.

For each of the models:

$$t_1(t+1) = t_{11}(t+1) + t_{12}(t+1)$$

$$t_{x1}(t+1) = t_{11}(t+1) + t_{21}(t+1)$$

$$D(t+1) = t_{11}(t+1) * t_{22}(t+1) - t_{12}(t+1) * t_{21}(t+1)$$

Model 2: maternal imprinting

All T1 offspring from a T1 mother are T11 individuals. T2 offspring from a T1 mother are T21. Likewise, all T1 offspring from a T2 mother are T12 individuals and T2 offspring from a T2 mother are T22. Bracketed numbers indicate the corresponding cells [row, column] of table 1.

$$t_{11}(t+1) = [1,1] + [2,1] + \tfrac{1}{2}[1,2] + \tfrac{1}{2}[2,2]$$

$$t_{12}(t+1) = \tfrac{1}{2}[3,1] + \tfrac{1}{2}[4,1]$$

$$t_{21}(t+1) = \tfrac{1}{2}[1,2] + \tfrac{1}{2}[2,2]$$

$$t_{22}(t+1) = [4,2] + [3,2] + \tfrac{1}{2}[3,1] + \tfrac{1}{2}[4,1]$$

Model 3: paternal imprinting

All T1 offspring from a T1 father are T11 individuals. T2 offspring from a T1 father are T21. Likewise, all T1 offspring from a T2 father are T12 individuals and T2 offspring from a T2 father are T22.

$$t_{11}(t+1) = [1,1] + [2,1] + \tfrac{1}{2}[3,1] + \tfrac{1}{2}[4,1]$$

$$t_{12}(t+1) = \tfrac{1}{2}[1,2] + \tfrac{1}{2}[2,2]$$

$$t_{21}(t+1) = \tfrac{1}{2}[3,1] + \tfrac{1}{2}[4,1]$$

$$t_{22}(t+1) = [4,2] + [3,2] + \tfrac{1}{2}[1,2] + \tfrac{1}{2}[2,2]$$

Model 4: oblique imprinting

All T1 individuals, regardless of their parents' traits, may imprint on either a T1 or a T2 individual. The chance that a T1 individual imprints on trait T1 is dependent on the frequency with which this trait occurs in their parents' generation.

$$t_{11}(t+1)=t_1^*\left([1,1]+[2,1]+\tfrac{1}{2}\left([1,2]+[2,2]+[3,1]+[4,1]\right)\right)$$

$$t_{12}(t+1)=t_2^*\left([1,1]+[2,1]+\tfrac{1}{2}\left([1,2]+[2,2]+[3,1]+[4,1]\right)\right)$$

$$t_{21}(t+1)=t_1^*\left([3,2]+[4,2]+\tfrac{1}{2}\left([1,2]+[2,2]+[3,1]+[4,1]\right)\right)$$

$$t_{22}(t+1)=t_2^*\left([3,2]+[4,2]+\tfrac{1}{2}\left([1,2]+[2,2]+[3,1]+[4,1]\right)\right)$$

2) The equilibria of models 1-4

We were able to solve for the equilibria in the phenotype matching and the oblique imprinting model. The phenotype matching model has 6 equilibria for t_1, the oblique imprinting model has 7. Three of the equilibria of each of the models are mentioned in the main body of the paper (t_1=0, t_1=1/2, t_1=1). The others are expressed in terms of a and s. These will generate complex, negative values, as well as positive values for t_1, depending on how ɑ and s are chosen. However, other than in a very small area of the parameter space discussed in the "Equilibria and Stability" section above, numerical simulations indicate there are no biologically relevant equilibria in these models other than the three already mentioned above.
We were not able to solve all of the equilbria of the maternal imprinting and paternal imprinting models. For maternal imprinting, there are at least 15 equilibria, and for paternal imprinting probably twice that number. However, these models also have the equilibria of t_1=0, t_1=1/2 and t_1=1, and simulations do not indicate the presence of any other biologically relevant equilibria.

3) Stability analysis of equilibria.

The stability of each equilibrium point was analysed with a linear stability analysis using Mathematica. The Jacobian matrix in each model was formed using the three recursion equations for t_1, t_{x1} and D. The leading eigenvalue of this matrix was then set equal to 1 (bifurcation point) and the resulting equation was solved for s, the level of positive frequency dependent selection that causes a switch of stability. This yielded an expression for s, with the strength of preference, ɑ, as a second variable. These expressions were used to compare the relative strength of frequency dependent selection, as a proxy for the ease of speciation, necessary in each model to reach a stable polymorphic equilibrium (see table 3). The preference gene model does not include positive frequency dependent selection, so this analysis was not performed on that model.

4) D in the preference gene model at line of equilibria.

The genetic linkage disequilibrium in the preference gene model was calculated, following Kirkpatrick 1982, using our modifications to this model (absence of natural selection, and $\alpha 1 = \alpha 2 = \alpha + 1$).

The equation for the $\hat{D}$ line is as follows:

$$\hat{D} = \frac{1}{2\alpha}\left(1 + \alpha p_1 + \alpha t_1 - 2\alpha p_1 t_1 - \sqrt{1 + 2\alpha p_1 + \alpha^2 p_1^2 + 2\alpha t_1 - 4\alpha p_1 t_1 - 2\alpha^2 p_1 t_1 + \alpha^2 t_1^2}\right)$$

Figure 2 shows the level of disequilibrium at three values of t_1 on the line of equilibria, dependent on α. The corresponding frequency of p_1 also depends on α, by the following equation:

$$p_1 = \frac{1 + t_1 \alpha}{2 + \alpha}$$

The curves for p_1 at three values of t_1 are shown in figure A-1.

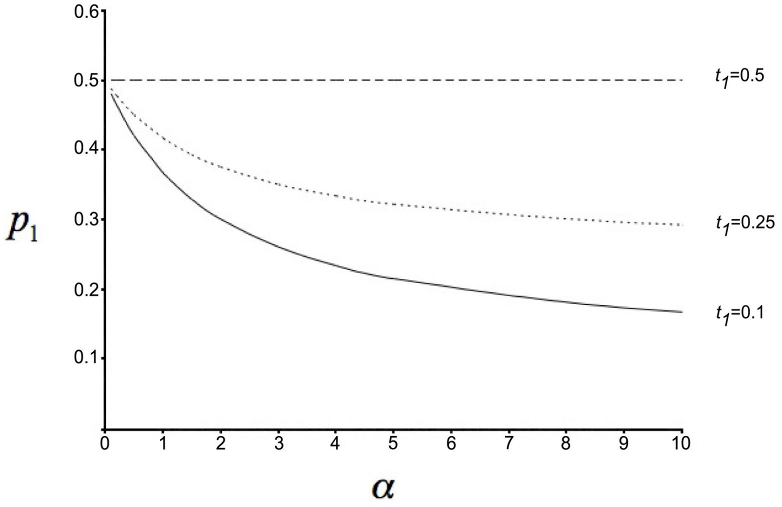

Figure A-1. Frequency of p_1 varying with the strength of preference, α, for three frequencies of t_1 on the line of stable equilibria.

Chapter 3

Early learning influences species assortative mating preferences in Lake Victoria cichlid fish.

Machteld N. Verzijden and Carel ten Cate

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Abstract

The Lake Victoria ‘species flock’ of cichlids is puzzling because reproductive isolation often occurs in the absence of substantial ecological differences among species. Theory predicts that this cannot evolve with most genetic mechanisms for mate choice. We provide the first evidence that learning, in the form of sexual imprinting, helps maintain reproductive isolation among closely related cichlid species. Using a cross-fostering experiment, we show that young females develop a sexual preference for males of their foster mothers’ species, even reversing species assortative mating preferences. We suggest that learning creates favourable conditions for reproductive isolation to evolve.

Key words: imprinting; mate choice; speciation.

Introduction

One of the least understood processes in speciation is the evolution of reproductive isolation without a geographical barrier. Though long thought implausible, speciation without geographical isolation recently has been suggested for a few cases, among which are the haplochromine cichlid fish (Seehausen & van Alphen 1999). Both Lake Malawi and Lake Victoria harbour large species flocks, endemic to each lake (Salzburger et al. 2005). Adaptations in jaw morphology may explain the radiation into various feeding niches (Kocher 2004). However, often several closely related species are found in sympatry with little morphological and ecological difference while a secondary sexual character, nuptial coloration, has strongly diverged (Seehausen & van Alphen 1999). This may be explained by disruptive sexual selection for conspicuous coloration (Maan et al. 2004) and strong assortative mating (Seehausen & van Alphen 1998). Postzygotic isolation cannot explain the maintenance of these species (Seehausen et al. 1997). In spite of great interest

in these species and their evolution, we still do not have a clear understanding of how they have evolved or maintain reproductive isolation. Learning by young individuals about the maternal phenotype as a model for their later sexual preference could promote reproductive isolation in sympatry. Recent models show that this allows evolution of assortative mating under a wider range of conditions than most scenarios involving a genetic background for mate preferences (Felsenstein 1981, chapter 2; Irwin & Price 1999). Such early learning of a sexual preference is known as sexual imprinting. It is prevalent in birds (ten Cate & Vos 1999) and is also documented in mammals (Kendrick et al. 1998). Females of haplochromine cichlid species show mouth brooding and continued protection of the brood after hatching. This might provide the offspring the opportunity to learn specific characters of their mother. A few studies have addressed imprinting in cichlids (Siepen & Crapon de Caprona 1986; Barlow et al. 1990) but so far no clear examples of parental sexual imprinting in fish are known. Sexual imprinting could promote the evolution of reproductive isolation, as it would strongly link phenotype to mate preference. Here, we test whether mate preferences in females of two Lake Victoria haplochromine cichlid species are affected by their mothers' phenotype. The closely related species pair *Pundamilia pundamilia* and *Pundamilia nyererei* is a model for the study of mate preferences and reproductive isolation in Lake Victoria haplochromine cichlids. They show only slight ecological and morphological differentiation and are sympatric throughout the range of *P. nyererei* (Seehausen & van Alphen 1999). Male nuptial coloration in *P. pundamilia* is mostly blue and *P. nyererei* red (figure 1). To test for early learning, we designed an interspecific cross-fostering experiment between the two *Pundamilia* species. The virgin daughters from these broods were tested for their preference for males of the two species.

Material and Methods

Housing and breeding
Wild caught *P. pundamilia* and *P. nyererei* from Makobe island, Tanzania, were housed in single species stock tanks (size 1x 0.4 x 0.6 m). For breeding, up to 12 females were housed with one male that was replaced regularly. All tanks were connected to a central recirculation water filter system. Water temperature was 24.58C, G18C, light regime was 12 L : 12 D. Fish were fed daily with fresh shrimp and peas or commercial pellets and flakes.

Cross-fostering and raising of the broods
Brooding females that had spawned approximately at the same time (maximum 4 days apart) were gently forced to spit out their eggs, within 2–5 days after spawning. Eggs were then taken up in a plastic pipette, which was then emptied in another female's mouth, such that each female received the eggs of the other female. All four types of crossings were done, both within and between species. Each treated female was placed in a visually isolated small tank. Mouth brooding takes 3–4 weeks, then the female releases the fry. The fry then start foraging independently, while females guard them for three weeks. When this stopped prematurely, the female was placed behind a perforated transparent sheet within the tank. Females were removed after four weeks. Each brood was later placed in a stock tank (1.5–5 months after spawning) exclusive for that brood. With first signs of nuptial coloration (average 170 days), we visually separated brothers and sisters with a perforated opaque sheet, to prevent them from gaining breeding experience. At sexual maturity, PIT tags (12 mm glass tags, UKID122GL Biomark, Inc.) were implanted in the left belly cavity. Females were then placed in a communal tank with conspecific females of other treatment broods.

Testing
Two grids (mesh size 160 x 160 mm) divided the experimental tank (2 x 0.5 x 0.5 m) into three equal compartments. The outer compartments contained a *P. pundamilia* and *P. nyererei* male. Bricks served as territorial 'rocks'. The males were matched in size (mean difference standard length 0.7 mm s.e.m. ±0.07, range 79–126 mm). Males were placed into the experimental tank 24 h before testing. In total, 34 *P. nyererei* and 36 *P. pundamilia* males were used, (re) combined into 53 pairs. Males performed on average in 2.48 tests (±0.19 s.e.m.). A female was placed in the middle compartment 30–60 min prior to testing while opaque screens hid the males from her. A trial started with removal of the screens and lasted 30 min. We tested the females blindly with regard to treatment. The focal female could interact freely with the males which were restricted to the outer compartments of the tank (figure 1). Males courted females when they entered their compartment. Males initiate courtship by a series of behavioural displays, to which females can respond by approaching. Courtship may then proceed and eventually lead to spawning. This was scored as described in Seehausen & van Alphen (1998). In a

successful trial, both males displayed at least two quivers and the female approached a quiver twice. Unsuccessful trials were repeated later on. We tested until each female had two successful trials, with different male pairs. Male species position (left or right) was reversed in the second trial. We calculated the relative approach ratio as follows: (number of approaches to conspecific male/number of quiver displays conspecific male)—(number of approaches to heterospecific male/number of quiver displays heterospecific male). In total, 43 females from 21 broods were tested. Five broods of each species were interspecifically cross-fostered, and five *P. nyererei* and six *P. pundamilia* were control-fostered broods. The data is presented in table 1 of the electronic supplementary material.

Colour analysis of males and females
Males and females were photographed with a Sony DSC-F707 camera at the same place under the same lighting conditions, with the same aperture each time. Pictures were analysed with Sigmascan Pro v 4.0 (SPSS Inc.; Maan et al. 2004). Only the dorsal, most intensely coloured part of the body was used for coloration analysis. Ranges for hue and saturation were maximized for differences between the males of the two species. Those same settings were then used in females. We analysed the red/blue ratio. All statistical analysis was done in R (R Development Core Team 2005) with nested Generalized Linear Models (GLMM), stepwise deleting factors from a fully saturated model until the minimal adequate GLMM was found, whose factors and significance levels are reported. Models were nested, correcting for any pseudoreplication; broods within treatment and individuals (two trials) within broods.

Results

The behaviour of females between two tests was significantly correlated: r=0.33, t_{41}=2.2, p=0.03. Species of foster mother had a significant effect on the preference of the females. Interspecifically cross-fostered females of both species preferred heterospecific males more than did females of the intraspecific crossfoster treatment (*P. nyererei*: GLMM, F(1,10)=4.77, p=0.0011; *P. pundamilia*: GLMM, F(1,9)=5.54, P=0.045; Both species: GLMM, F(1,19)=21.39, p=0.0002, figure 1). Females in three treatment groups showed significant preferences for males of their foster mothers' species (interspecific treatment *P. pundamilia* and *P. nyererei*: p=0.0003, t_{12}=5.02; p=0.0012, t_9=4.63, respectively, and intraspecific treatment *P. nyererei*: p=0.038, t_9=2.41, nested one-sample t-tests). In trials with *P. pundamilia* females, conspecific males displayed significantly more than heterospecific males: F(1,84)=29.26, p<0.001. Since the number of female approaches best fitted an inverse exponential relation to male display effort (see figure 2, appendix), this lowered their preference scores. The ratio red/blue was significantly different between the females of the two species (p<0.001, F (1,60)=23.79). More results are given in table 2 in the appendix.

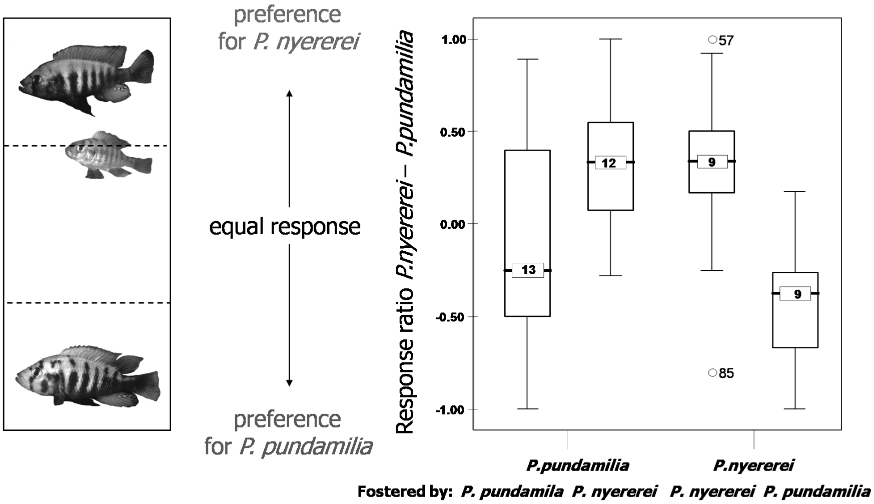

Figure 1. The cross-fostering experiment. Left: The two-way female mate choice experimental setup. Females can enter male territories through grids, while the larger males cannot leave theirs (see colour pictures on foldover of the cover). Right: The results of the cross-fostering experiment. For this figure, we subtracted the approach ratio to PP from PN. Scores above zero reflect a higher approach ratio to *P. nyererei* males and scores below zero reflect a higher approach ratio to *P. pundamilia* males. Graphs represent median, interquartile ranges and full ranges. Numbers on median bars show number of individuals in each treatment group.

Discussion

This experiment provides the first strong evidence for cichlids, and for fish in general, that females prefer males of the maternal phenotype as a result of imprinting. In contrast, studies testing for sexual imprinting in substrate spawning Central American cichlids (Siepen & Crapon de Caprona 1986; Barlow et al. 1990) have shown small and inconsistent effects. This may indicate that the ability to imprint has evolved in consort with the mouth brooding in haplochromine cichlids, or that a latent ability for sexual imprinting is expressed with the opportunity provided by the mouth-brooding behaviour. Nuptial coloration is an important cue in both interspecific (Seehausen & van Alphen 1998) and intraspecific mate choice (Maan et al. 2004), suggesting its importance in reproductive isolation. While sexually mature males are brightly coloured, females are mostly yellow or light brown. However, the females do show some differentiation in colour in the same direction as males (table 2 of electronic supplementary material), which therefore may provide a basis for the learnt preferences. Interestingly, visual early learning was also shown to mediate assortative shoaling preferences in zebra fish, *Danio rerio* (Engeszer et al. 2004).

Olfactory cues are used in broodcare in parent–offspring communication in cichlids (Kühme 1964). These may be correlated with male coloration (Plenderleith et al. 2005), or reflect species differences, possibly through MHC and related mechanisms (Zavazava & Eggert 1997). This could provide species specific cues to the hatching brood. Imprinting on olfactory cues is therefore also plausible. As both colour and chemical cues could be used at all stages of the experiment, we cannot conclude which cue served as imprinting stimulus.

In a sympatric speciation scenario, disruptive sexual selection on coloration may have initiated divergence of mating cues (Maan et al. 2004) but this alone may not provide full reproductive isolation. However, imprinting can be a very effective mechanism in linking mate preferences to other diverged characters (chapter 2).

In summary, we show that cichlid young imprint on their mothers' phenotype and that this can reverse species assortative mating preferences. All haplochromine cichlids in the East African great lakes are maternal mouth brooders. Imprinting may be widespread among them, given their high degree of relatedness. We suggest that imprinting greatly enhanced their tendency for assortative mating. The presence of imprinting may therefore be critical to explaining the many sympatric species of haplochromine cichlids.

Acknowledgments

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Appendix

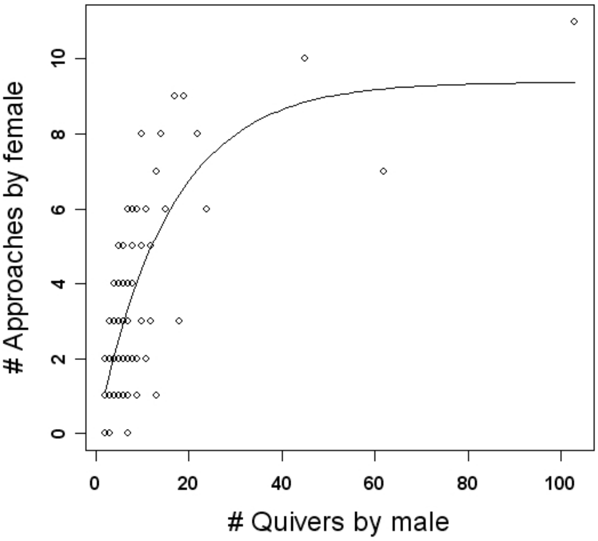

Figure 2. The relation between the number of quivers given by a male in a trial and the number of approaches of the female to that male. When a female's tendency to approach after a display by the male decreases with an increase in male display behaviour this would be reflected in a non-linear relationship between # quiver displays made by the males and # approach of the female to that male. This is exactly what we find, because their relationship can be best described by an inverse exponential function, which we fitted with the following parameters:

Fit of the model:

"# approach of female" = 9.3[1-exp(-0.06 * "# quivers")].

Details of the estimates in this fit:

Estimate	Std. Error	t value	Pr(>|t|)
9.390644	0.806215	11.648	< 2e-16 ***
0.063310	0.007978	7.935	2.7e-13 ***

	Males (n=10 *P. pundamilia*) (n=10 *P. nyererei*)		**Females** (n=23 *P. pundamilia*) (n=39 *P. nyererei*)	
	Red	**Blue**	**Red**	**Blue**
P. pundamilia	6.1% ±0.02	7.6% ±1.02	0.1% ±0.02	3.1% ±0.66
P. nyererei	65.8% ±6.9	0.6% ± 0.40	0.8% ±0.33	1.6% ±0.28
	Ratio red/blue P<<0.001 F(1,18)= 146.4		**Ratio red/blue** P<0.001 F (1,60)=23.8	
P. pundamilia	0.02 ± 0.004		0.15 ±0.052	
P. nyererei	583.2 ±137.5		2.4 ±1.05	

Table 1 Colour analysis of males and females.
Numbers are the percentage dorsal body area that was red or blue (± S.E.) of males and females of both species and the ratio red/blue scores of both species.

				Test 1				**Test 2**			
				***P.nye* male**		***P.pun* male**		***P.nye* male**		***P.pun* male**	
Species	**Foster mother**	**Brood**	**Fe- male**	**Q mad e**	**App r to Q**	**Q mad e**	**App r to Q**	**Q mad e**	**Appr to Q**	**Q made**	**Appr to Q**
P. nye	*P. nye*	L	D55D	4	1	6	5	2	2	2	0
P. nye	*P. nye*	M	EA0B	6	3	4	1	2	2	13	1
P. nye	*P. nye*	N	E34E	2	2	18	3	7	6	22	8
P. nye	*P. nye*	L	E8FE	8	4	7	0	19	9	4	1
P. nye	*P. nye*	O	E07C	2	0	5	4	2	2	5	2
P. nye	*P. nye*	O	DA0B	3	1	7	2	4	2	2	0
P. nye	*P. nye*	P	C80C	2	1	11	2	4	2	2	0
P. nye	*P. nye*	O	E6DD	5	1	3	1	2	1	4	1
P. nye	*P. nye*	O	CF12	6	3	4	3	5	3	3	1
P. nye	*P. pun*	Q	D902	13	7	5	4	2	0	3	2
P. nye	*P. pun*	R	E881	5	2	4	3	5	1	17	9
P. nye	*P. pun*	R	DBA1	4	2	13	7	3	1	3	2
P. nye	*P. pun*	R	F1A1	2	1	7	3	15	6	5	4
P. nye	*P. pun*	S	A1D9	2	0	11	6	9	1	3	3
P. nye	*P. pun*	T	E882	3	2	6	3	2	0	2	2
P. nye	*P. pun*	U	E498	3	0	2	2	2	0	5	2
P. nye	*P. pun*	U	CDD0	3	1	3	2	2	0	6	4
P. nye	*P. pun*	T	B28E	2	0	8	4	2	2	2	2
P. pun	*P. pun*	A	C8A6	2	2	12	5	3	3	6	3
P. pun	*P. pun*	B	D123	2	2	10	3	6	5	6	3
P. pun	*P. pun*	A	DBC7	10	8	24	6	2	0	6	2
P. pun	*P. pun*	B	E5C2	2	2	5	3	2	2	62	7
P. pun	*P. pun*	C	E046	5	1	5	3	2	1	4	3
P. pun	*P. pun*	D	D8A4	4	1	3	3	10	5	2	2
P. pun	*P. pun*	C	DBF1	7	4	3	0	3	1	7	6
P. pun	*P. pun*	C	E1C7	2	0	3	3	5	1	9	6
P. pun	*P. pun*	C	CC4B	9	1	8	5	4	1	14	8
P. pun	*P. pun*	D	D70C	5	2	10	5	2	1	2	2
P. pun	*P. pun*	D	EBFE	8	2	6	4	7	3	5	4
P. pun	*P. pun*	D	EAFF	2	1	8	6	3	0	3	2
P. pun	*P. pun*	E	CA0F	5	1	6	5	2	1	4	3
P. pun	*P. nye*	F	CD8F	4	4	4	2	4	3	3	2
P. pun	*P. nye*	F	D18E	2	1	103	11	5	4	5	1
P. pun	*P. nye*	G	D815	2	2	2	0	2	2	2	2
P. pun	*P. nye*	H	DBA2	5	5	7	2	5	3	4	3
P. pun	*P. nye*	I	E480	3	3	13	7	2	2	12	3
P. pun	*P. nye*	J	EFD8	7	3	3	2	5	4	6	2
P. pun	*P. nye*	H	F0FF	3	2	45	10	9	2	6	3
P. pun	*P. nye*	J	F124	3	2	5	3	2	2	7	2
P. pun	*P. nye*	F	DEDA	4	2	18	3	4	3	7	1
P. pun	*P. nye*	I	EAE7	4	2	2	1	4	2	4	1
P. pun	*P. nye*	K	E8D1	3	2	4	2	2	2	3	2
P. pun	*P. nye*	K	EC9C	5	3	2	0	2	1	6	1

Table 2

Raw data obtained from mate choice trials.

P.nye = *Pundamilia nyererei*	Q = quiver display
P.pun = *Pundamilia pundamilia*	Appr = approach

Chapter 4

Cross-fostering does not influence the mate preferences and territorial behaviour of male Lake Victoria cichlid fish.

Machteld N. Verzijden, Juul Zwinkels, Carel ten Cate

Abstract

The ability to recognise conspecifics in contexts of mate choice and territorial defence may have large effects on an individual's fitness. Understanding the development of assortative behaviour may shed light on how species assortative behaviour evolves and how it may influence reproductive isolation. This is not only the case for female mate preferences, but also for male mate preferences and male territorial behaviour. Here we test with a cross-fostering experiment whether early learning influences male mate preferences and male – male aggression biases in two closely related, sympatrically occurring cichlid species *Pundamilia pundamilia* and *P. nyererei* from Lake Victoria. Males that had been fostered, either by a conspecific female or a heterospecific female, 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.

Key Words: Aggression, fish, male mate choice, *Pundamilia pundamilia*, *Pundamilia nyererei*, reproductive isolation, rival imprinting, speciation, sexual imprinting, territoriality.

Introduction

Understanding how new species evolve and persist is a key issue in evolutionary biology. One of the more controversial issues is how this may happen while the (incipient) species co-occur. This process of sympatric speciation has long been thought of as improbable, but recent empirical work has revealed several possible cases (e.g. Bush 1969; Schluter & McPhail 1992; Seehausen & van Alphen 1999; Kornfield & Smith 2000; Korol et al. 2006). Sympatric speciation might occur under a number of critical conditions, and the developmental mechanisms for mating preferences and aggression biases may have direct bearing on a number of them. First, reproductive isolation should arise very quickly between the diverging phenotypes (reviewed in Kirkpatrick & Ravigne 2002). This means that individuals should be mating assortatively, mediated by their mating preferences. If both sexes have assortative mate preferences, this could strengthen the assortative mating pattern (Almeida & de Abreu 2003; van Doorn et al. 2004). Mutual mate choice is commonly found in species where both sexes substantially invest in the offspring (i.e. Amundsen 2000). Although male mate preferences are not a priori predicted in polygynous mating systems it is found in several polygynous (review in Amundsen 2000), and even in lekking bird and fish species (Saether et al. 2001; Werner & Lotem 2003; Pierotti & Seehausen 2007). It may thus occur more often than previously thought. Mating preferences are assortative if individuals prefer mates with a similar phenotype as themselves. Therefore, the critical question is how mating preferences arise. Especially in a population with rapidly changing phenotypes, the extent of assortative mating may be quite different between different developmental mechanisms (Kirkpatrick 1982; Laland 1994; ten Cate 2000, this thesis chapter 3).

A second critical condition is that the coexistence of both incipient species can occur. This may be possible under negative frequency dependent selection (e.g. reviewed in van Doorn et al. 2004; Rueffler et al. 2006). Such selection promotes invasion of a new phenotype into the population, and at the same time allows stable coexistence of two phenotypes. Male-male aggression may yield such selection when males direct their aggression strictly against males of a similar phenotype, giving males of a rare phenotype the advantage of having fewer aggressive interactions (Mikami et al. 2004; Seehausen & Schluter 2004; van Doorn et al. 2004). This may for instance occur when males that compete for the same females show more interest in chasing each other away from their mating grounds than in chasing males competing for different females. Because aggressive interactions are likely to be costly, selective aggressive behaviour has a direct bearing on the fitness of an individual. Therefore, aggression biases towards males that compete for the same females or the same resources (i.e. conspecific males), may be adaptive in the sense that this avoids unnecessary competitive interactions. Aggression is often found to be directed more at conspecifics. In birds, for instance, males respond more to conspecific song than to sympatric heterospecific song (reviews in Becker 1982; Irwin & Price 1999), túngara frogs respond indiscriminate to allopatric heterospecific calls (Bernal et al. 2007), and in fish males also respond more to conspecific territorial intruders (Genner et al. 1999b; Dijkstra et al. 2006b). As for mate preferences, the question is how the direction of aggression develops, because this critically influences the degree of assortative aggression.

Because of its central role, the study of developmental mechanisms for species recognition will improve our understanding of how the degree of assortative behaviour (mating, aggression) changes with new circumstances, and how assortative mating patterns can arise. It could ultimately also give us insight in how this behaviour influences speciation processes.

One mechanism that may promote assortative mating particularly is sexual imprinting (Laland 1994; Irwin & Price 1999; ten Cate 2000, this thesis chapter 2). Sexual imprinting is a form of learning, in which young animals learn about the phenotype of an individual, usually a parent, and use this as a model for future sexual preferences. It is a well documented phenomenon, especially in birds (reviewed in ten Cate & Vos 1999), but also in other taxa (Kendrick et al. 1998; Bereczkei et al. 2004, this thesis chapter 3), and has recently gained attention in the context of reproductive isolation. Sexual imprinting may aid the evolution of assortative behaviour in two ways. First because it immediately provides preferences for newly arising phenotypes (ten Cate & Vos 1999; Irwin & Price 1999) and, second, because it ensures a strong link between the phenotype of the individual and its species assortative behaviour (Laland 1994; ten Cate & Vos 1999; Albert 2005, this thesis chapter 2).

Mate preferences might also develop by learning at a later stage in life through (sub) adult experience (e.g. Dugatkin & Godin 1992; Schlupp & Ryan 1997; Hebets 2003). Alternatively, different mating preferences may be predominantly determined by genetic differences (e.g. Shaw 2000; Ritchie 2000). All these developmental mechanisms are not entirely mutually exclusive. They vary however in the degree of assortative behaviour they may mediate. Learning behaviour at a later stage in life may not produce preferences for phenotypes similar to an individual's own phenotype. This is because learning will often take place through experience with individuals that are relatively unrelated, which hence may not share the same phenotype or genotype. When genetic differences account for the developmental differences in preferences, these will in general not be very plastic. This means that in populations with quickly evolving phenotypes, preferences may lag behind and hence will not be assortative.

Imprinting might also link the direction of aggression towards males of a similar phenotype as themselves. Two studies on bird species demonstrated such imprinting, (Vos 1994; Hansen & Slagsvold 2003), coined 'rival imprinting' by Hansen & Slagsvold. However, a central American cichlid showed no evidence of such imprinting (Barlow & Siri 1987).

It is possible that there is a relation between a male's mate preference and his aggression bias. Such a bias may be internally coupled, for instance through a genetic linkage, when males' aggression biases and mate preferences are both genetically determined. It may also be that learning in one context, for instance learning which male is a rival, has consequences for a males' mate choice. Experience in one context, indirectly influences a male's mate preferences. Such a coupling of behavioural biases would then be genetically determined, but the biases themselves may not have to be. Finally, unrelated experiences in aggressive and mate choice contexts may also yield high degrees of assortative behaviour in both contexts, and thus a relation in the behaviour between the two contexts.

Case studies for sympatric speciation are the East African cichlid species flocks. East African lakes harbour up to 1100 endemic cichlid species (conservative estimate, Turner et al. 2001). The observation that many sympatric species pairs differ in male nuptial coloration, but are morphologically very similar, has fostered the hypothesis that sexual selection was a major driving force in their speciation (Seehausen & van Alphen 1999; Kornfield & Smith 2000). Male cichlids defend territories vigorously against other males in a lek-like aggregation in order to attract and court females and eventually mate with them (Maan et al. 2004; Seehausen & Schluter 2004). The sympatric species pair *Pundamilia pundamilia* and *P. nyererei* is studied as a model for speciation research in Lake Victoria cichlids. Males of these species, originating from wild populations, are known to show more aggression towards a conspecific intruder than to a male intruder of the sister species (Dijkstra et al. 2006b). Also, female mate choice was found to be assortative with regard to species identity (Seehausen 1997; Seehausen & van Alphen 1998).

In a previous paper, we demonstrated evidence that female mate preferences are mediated by sexual imprinting on their mother's phenotype (chapter 3). In this study, we focus on the mechanisms that mediate the male-male aggression biases and male mate preferences in these species. Males of both *P. nyererei* and *P. pundamilia*, were raised in an interspecific cross-fostering experiment, after which each male was tested for its species assortative behaviour in two contexts, territorial defence and mate choice. With these experiments we tested the following questions. First, is male territorial defence mediated by imprinting on the maternal phenotype, like that of the females? Second, are male mate preferences, if there are any, mediated by sexual imprinting? We examine the degree of assortative behaviour of the males in the two different contexts, and we also compare the results for male mate choice with those for females, as presented earlier (chapter 3). The implication of the results for our understanding of speciation in these species is discussed.

Methods

Housing and Cross-Fostering of the Animals

Wild caught *P. pundamilia* and *P. nyererei* from Makobe island, Tanzania (Seehausen & Bouton 1997), were housed in single species stock tanks (size 1 x 0.4 x 0.6 m). For breeding, up to 12 females were housed with one male that was replaced regularly. All tanks were connected to a central recirculation water filter system. Water temperature was 24.5 +/- 1° C; the light regime was a 12 :12 h light:dark cycle. Fish were fed daily with fresh shrimp and peas or commercial pellets and flakes. Brooding females that had spawned approximately at the same time (maximum 4 days apart), were gently forced to spit out their eggs, within 2-5 days after spawning. Eggs were then taken up in a plastic pipette, which was then emptied in another females' mouth, such that each female received the eggs of the other female. All four types of egg exchanges were done: both within and between species. Each treated female was placed in a visually isolated small tank (25x25x10 cm). Mouth brooding takes 3-4 weeks, then the female releases the fry. Fry then start foraging independently, while females guard them during 3 weeks. This occasionally stopped prematurely, in which case the female was placed behind a perforated transparent sheet within the tank, maintaining both visual and olfactory communication. Females were removed after 4 weeks. At 1.5 to 5 months after spawning, each brood was placed in a stock tank exclusive for that brood. With first signs of nuptial coloration (average 170 days), we visually separated brothers and sisters with a perforated opaque sheet, to prevent them from gaining breeding experience. At sexual maturity, PIT tags (12 mm glass tags, UKID122GL Biomark Inc., Idaho, USA) were implanted in the left belly cavity. Males were then placed in a communal tank with conspecific males of other treatment broods. Each male was first tested twice for aggression bias towards each of the two species, and then twice for mate preference, so that each male was tested four times. The inter-test interval was at least one week. All tests were done blindly with respect to the foster-treatment of the male. The wild caught parental generation was kept in our facilities for further breeding.

Male Behaviour

Adult males defend territories in order to attract females. Territories in these species are only for reproduction, feeding occurs outside the territories (Seehausen & Schluter 2004). Aggressive interactions usually take place at the border of the territories, to repel intruders. We recorded four types of behavioural displays during aggressive interactions (Baerends & Baerends-van Roon 1950; Dijkstra et al. 2006b): Frontal displays, bites, lateral displays and quivers. During frontal displays, males line up head to head. During bites, males grab each other's mouth and pull back and forth. This is usually preceded by a frontal display. In our set-up males could not physically bite each other, but they bit the Plexiglas separating them (see below). We added the number of times for frontal displays and bites into one measure (abbreviated with FD-B), as some males never performed bites and some never performed frontal displays prior to attacking, while others performed both. During a lateral display, the male extends his dorsal, anal and pelvic fins, and positions itself such that its flank is in front of the head of the opponent. The quiver, finally, is usually preceded by a lateral display. The male has the same position as in the lateral display, and the fins are equally extended, but a fast shaking movement of the body is made. Lateral displays and quivers are also sequentially connected behaviours, and again, some males never performed quivers or lateral displays. We therefore took lateral displays and quivers as one measure also (abbreviation: LD-Q).

Lateral displays and quivers are also used in courtship (Baerends & Baerends-van Roon 1950; Seehausen & van Alphen 1998). The difference in the displays between the two contexts is that during aggressive interactions, the operculi are opened and the lower jaw is held at a wider angle. This gives the appearance of a broader head. During courtship, the approach of a male to a female may start a sequence of displays, starting with a lateral display, followed by a quiver and a lead swim and potentially ending in spawning. Females may respond to these behaviours by approaching the male and following him to the centre of his territory, although in our setup females could not fully complete the follow to a lead swim (see below). We scored the number of lateral displays, quivers and lead swims in the mate preference tests. We analysed male courtship behaviour per display sequence. We also noted the time the focal individual spent with each male or female. In both experiments, we used Observer 3.0 (Noldus information technology, Wageningen, The Netherlands).

Male Aggression Tests

The focal male was placed in the experimental tank (1x0.4x0.5 m) 48 hours prior to testing. Each male also had a 'neighbour male': a smaller male of a different Lake Victoria cichlid genus placed at one end of the tank behind a Plexiglas sheet. We placed this male in the experimental tank to enhance territoriality (Dijkstra et al. 2006b). At the time of testing, two cylinders were placed in the experimental space of the focal male. One stimulus male was in each cylinder, making up a stimulus pair, with one male of each species. Stimulus males were matched in standard length (mean difference +/- SE = 0.4 +/- 0.1 mm). Observations started right after placement of the cylinders, and lasted until at least 5 minutes of aggressive interactions had passed. Each male was tested twice, and male intruder species position was reversed in the second test. We tested 7 *P. pundamilia* control males, 15 *P. pundamilia* cross-fostered males, 6 *P. nyererei* control males and 12 *P. nyererei* cross-fostered males, from in total 19 broods. In total we performed 80 aggression tests. As stimulus males we used 26 *P.pundamilia* males and 29 *P.nyererei* males, which were combined to 35 stimulus pairs. A focal male never encountered the same stimuli in the second test.

Male Mate Choice Tests

The focal male was placed in the experimental tank (2x0.4x0.5) 24 hours prior to testing. Also, at both ends of the tank, a Plexiglas sheet was placed, behind which a smaller male from a different genus was placed. This was again done to enhance territorial behaviour in the focal male. The reason for using two males in this case was to prevent side preferences, which might arise from the larger experimental space. We placed two 6-sided Plexiglas cylinders with 5 mm holes at equal distance from the centre of the tank. Water flow was directed into each of the cylinders. We placed one female into each cylinder, and the two females formed a stimulus pair, with one female of each species. Before the male was placed in the experimental tank, 6-sided opaque PVC cylinders were placed around the slightly smaller Plexiglas cylinders, to hide the females from the males until the time of testing (24 hours later). We used 43 *P. pundamilia* and 34 *P. nyererei* females as stimulus, which were recombined into 51 pairs. Females were matched for weight (mean difference +/- SE = 0.1 +/-0.2) and standard length (mean difference +/- SE= 0.2 g +/- 0.1). Weight and standard length were combined in one measure to reflect body condition, by dividing standard length by weight. Tests began by removing the opaque PVC cylinders and lasted 30 minutes. Female species position was reversed in the second test. We tested 4 *P. pundamilia* control males, 14 *P. pundamilia* cross-fostered males, 5 *P. nyererei* control males, 11 *P. nyererei* cross-fostered males, from in total 18 broods. A focal male never encountered the same stimuli in the second test.

Ethical Note

The wild-caught stock was collected by angling and gill netting after which the fish were housed in large tanks at TAFIRI (Tanzanian Fisheries and Research Institute) in Mwanza, Tanzania. The gill netted fish were taken out of the net under water as soon as they were caught in the net by scuba divers. All the fish that were captured survived and the angle punctures in the mouth/lips and gill net marks on the dorsum healed within a few days. Fish required no medical treatment. The number of fish collected at Makobe island, 20 of each sex and each species, could not have a significant effect on population sizes, which are estimated at several thousand. Before transport to The Netherlands (by air), the fish were packed in plastic bags provided with water and pure oxygen. Fish were inspected by a Veterinarian (Fish Technologist) before transport. The Ministry of Natural Resources and Tourism in Mwanza provided an export permit, number FS/L.80/1. The fish were imported into The Netherlands under licence 250-92/257 of the National Museum of Natural History in Leiden, The Netherlands, granted by the Dutch Tax and Customs Administration. The fish arrived on 16 February 2003 in Leiden, and were moved directly to our aquarium facilities. The whole transport procedure lasted 32 hours. None of the fish died during transport or after arrival in the laboratory.

The PIT tags were implanted by inserting a hollow needle slightly smaller in diameter than the tags (which are just less than 2 mm in diameter) into the abdominal cavity and then inserting the tag into the puncture. We did not use the implantation devise recommended by the manufacturer, because those punctures are unnecessarily large. The wound was sealed with paraffin paste. The procedure took place above water and lasted up to one minute. The implantation of the PIT tags did not cause any adverse effects on the fish. We did not observe any effects on behaviour, reproduction and nuptial colouration and the small puncture wounds healed quickly without further medical treatment. We did not use anaesthetic during implantation of the tags, because in a pilot study, we found that the effect of anaesthetic lasted several days

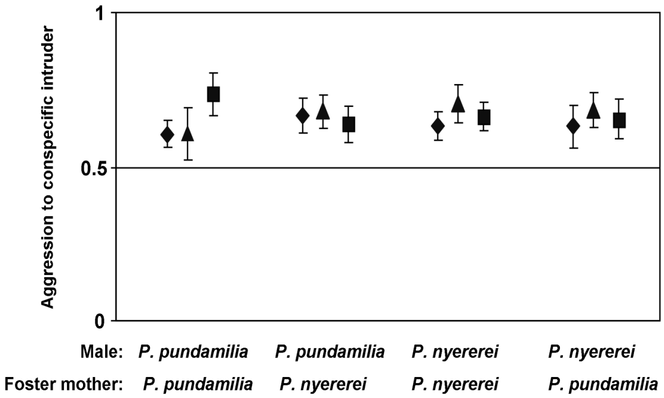

Figure 1 Male aggression test. Average proportion of aggressive behaviour directed at conspecific intruder. Diamond: time; Triangles FD-B; Squares: LD-Q. Mean +/- SEM. The horizontal line indicates equal response scores. There were no differences between the species, or treatments and all measures indicated a bias for aggression towards the conspecific intruder.

(assessed on swimming, eating and social behaviour), while unanaesthetised fish showed normal behaviour within minutes after the procedure.
During the aggression tests, males never had direct contact, and could therefore not inflict wounds on each other. Males did bump into the Plexiglass cylinder and attempted biting it as well. We did not observe any wounds or bruises on their mouths, and all males continued eating after the experiment, indicating that there were no adverse effects on their teeth and jaws. The University Committee for Animal Experiments (UDEC) approved this experiment under license number: DEC03079**.**

Statistical Analysis

All statistical analysis was done in R (R Development Core Team 2005). We fitted Generalised Linear Mixed Effect Models (GLMMs). All models were hierarchically nested, correcting for any pseudoreplication: broods within treatment, and individuals (two trials) within broods. For the analysis of the aggression biases, we had two fixed effects which each had two levels: species of the focal individual (*P.pundamilia* or *P.nyererei*) and its treatment (conspecific or heterospecific foster mother). If the fixed effect of species is significant in the model, the species respond differently in the experiments, if the fixed effect of treatment if significant, the males in the different treatments respond different in the experiments. An interaction would indicate that the treatments had a different effect in each species. In the analyses we included only one fixed factor 'species', but included both nested random effects 'brood' and 'individual'. We stepwise deleted factors from a fully saturated model until the minimal adequate GLMM was found, but always keeping repeated measures for each individual as a random factor. The factor significance levels are reported from a Chi-square test on the deviance, as appropriate for binomial data. When the minimal adequate model only included the intercept as a fixed effect (which indicates a deviation from an equal response to both stimuli), the intercept estimate is also reported.

For the aggression tests we fitted models to each of the following variables: the proportion of frontal displays and bites (FD-B) to conspecific males (relative to heterospecific males); the proportion lateral displays and quivers (LD-Q) to conspecific males; and the proportion of time spent interacting with conspecific males. For the male mate choice tests we fitted models to both the proportion of courtship events directed towards conspecific females and the proportion of the time spent with conspecific females. Because each male was tested twice in each experimental setup, we also tested for an effect of experience with that context, i.e. whether their behaviour changed between test 1 and 2.

We also tested for a relationship between aggression bias and mate choice preference of the individuals. We had data for this analysis from 15 *P. nyererei* and 18 *P. pundamilia* males; one of the *P. nyererei* males tested for mate choice was not tested for aggression bias and was therefore excluded from this analysis. For this analysis we used the average of the two tests in each setup (aggression and mate choice). Both the proportion of courtship displays to conspecific females and the proportion of time spent with conspecific females were regressed separately against the proportion of each of the two measures of aggressive displays (FD-B and LD-Q), and also against the time spent in aggressive interaction with conspecific males.

Results

Results Aggression Tests

Figure 1 shows that males of both species directed more aggression towards conspecific intruders than to heterospecific intruders. It also shows the absence of effect of the cross-fostering treatment. The full model we tested included the fixed effects: species, treatment and standard length difference. The latter was the difference in body length between the stimulus males. There was a strong preference for displaying, in both behavioural measures, towards the conspecific intruder, and also more time was spent with the conspecific intruder: fraction FD-B $F(1,39)=36.77$, $P<0.0001$; LD-Q $F(1,39)=17.68$, $P<0.0001$; time spent interacting with conspecific male: $F(1,39)=16.49$, $P<0.0001$. There was no effect of the standard length difference between the males of a stimulus pair. We found no effect of either the cross-fostering treatment or of species. See table 1 for the results and estimates of effect sizes from the GLMM.

Testing for the difference in behaviour between the two trials for each male, we found that males spent significantly more time attacking conspecific males in the second trial than in the first trial in both behavioural measures (FD-B: $F(1,78)=11.63$, $P<0.001$ with effect size 0.71 +/- 0.06 SE. LD-Q: $F(1,78)=3.3$, $P=0.05$ with effect size 0.63 +/- 0.07 SE) and time ($F(1,78=4.0$, $P=0.03$ with effect size 0.62 +/- 0.01 SE). Figure 3a shows the change between trials for the proportion time spent interacting with the conspecific intruder.

Results Male Mate Choice Tests

Figure 2 shows that it is unlikely that male mate choice was affected by the cross-fostering treatment. The main aim of this study is to address the effect of cross-fostering on male mate preferences. Due to the low sample sizes for the control groups, the statistical analysis has limited power. We therefore omitted a test for species differences in this treatment. The full model we tested included the fixed effects: treatment and body condition of the female (standard length / weight). Table 2 shows the results and estimates of effect sizes from the GLMM. We did not find an indication that the cross fostering treatment had any effect on the males' mate preferences. We found no effect of the difference in body condition within the stimuli pair in either the males' display behaviour or on the time the male spent with either member of the

	Explanatory variable	**Effect size**	**± s.e.**	**F**	**d.f.**	**p**
Full model: species x treatment + standard length difference, nested for individuals and brood						
Number of individuals: 40, from 19 broods						
FD-B	**different from equal**	0.72	0.16	36.77	1,39	**<0.0001**
	standard length difference	0.20	0.15	1.61	1,38	0.21
	treatment	0.19	0.33	0.25	1,37	0.62
	species	0.10	0.31	0.02	1,36	0.88
	species x treatment	0.19	0.77	0.06	1,35	0.80
	brood					0.31
LD-Q	**different from equal**	0.66	0.15	17.68	1,39	**<0.0001**
	standard length difference	0.13	0.16	0.61	1,38	0.44
	species	0.17	0.31	0.26	1,37	0.61
	Treatment	0.19	0.34	0.17	1,36	0.68
	species x treatment	0.23	0.70	0.10	1,35	0.75
	brood					0.28
time	**different from equal**	0.64	0.14	16.49	1,39	**<0.0001**
	standard length difference	0.06	0.13	0.20	1,38	0.66
	Species	0.06	0.28	0.04	1,37	0.85
	Treatment	0.02	0.31	0.01	1,36	0.99
	species x treatment	0.03	0.66	0.01	1,35	0.96
	Brood					0.13

Table1 Aggression analysis results of the GLMM.

Effects are listed in reverse order of removal from the model. In bold the final model, which only includes significant effects. When none of the fixed factors was significant (which was the case for each of the three variables), the analysis shows whether the variable is significantly different from equal response to both intruders. The estimates of the proportion behaviour (or time) were in all three variables significantly different from 0.5, indicating a bias towards interacting with the conspecific intruder.

stimulus pair. Interaction between female preferences for conspecific males and male preferences may result in an absence of a difference between treatments within a species. We therefore tested if females approached less to displays of heterospecific males. We found that there was no difference in the tendency to approach if the female was heterospecific or conspecific (pairwise T test t_{61}=0.338 *P*=0.736).

Since there was an absence of effect of treatment, we merged the data of the two treatment groups per species, and tested for a mate preference. There was, a hint for mate preferences in *P. nyererei* males, which displayed significantly more often to *P. nyererei* females than to *P. pundamilia* females: *F*(1,15)=9.58, *P*=0.006, estimate of proportion of displays to conspecific females 0.63 +/- 0.04 SE; and showed a trend to spending more time with *P. nyererei* females: *F*(1,15)=3.25, *P*=0.09, estimate of proportion of time spent with conspecific females: 0.55 +/- 0.03 SE. For *P. pundamilia* males, we did not find any differences in courtship behaviour or time spent with regard to the species of the stimulus females. Displays *F*(1,17)=0.13, *P*=0.72, estimate of proportion of displays to conspecific females 0.48 +/- 0.06 SE. Time spent: *F*(1,17)=1.76, *P*=0.20, estimate of proportion of time spent with conspecific females: 0.46 +/- 0.04 SE.

Testing for a change in behaviour between the first and the second trial, we found a significant interaction between trials effect and species, for both the display and time spent parameters. Display: species x trial *F*(1,64)=5.47, *P*=0.02; time: species x trial *F*(1,64)=15.68, *P*<0.001. This indicates that the males of the two species changed their behaviour differently between the two trials. To test for the main effect of trial, without an interaction effect, we tested per species separately. This showed that *P. nyererei* males increased their preference for conspecific females in the second trial compared to the first trial: displays *F*(1,30)=5.22, *P*=0.02, effect size: 0.13 +/- 0.05 SE, time spent: *F*(1,30)=13.31, *P*<0.0001, effect size: 0.07 +/- 0.02 SE. *P. pundamilia*

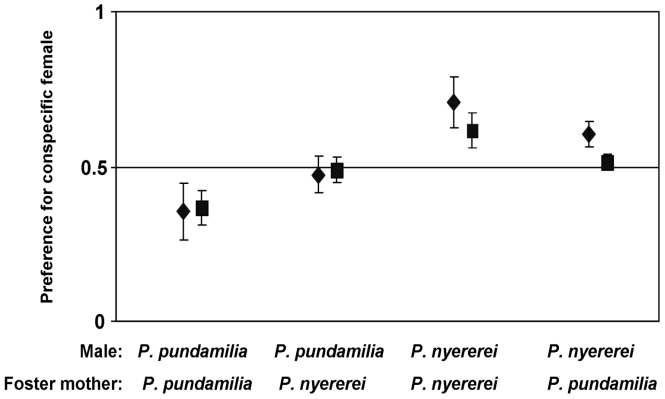

Figure 2 Male preference test. Average proportion of time and displays with conspecific female. Diamonds: displays; Squares:time. Mean +/- SEM. The horizontal line indicates equal response scores. We found no effect of treatment. There was a difference between the species: *P. nyererei* showed a preference for conspecific females, whereas *P. pundamilia* did not.

	Explanatory Variable	Effect size	± s.e.	F	d.f.	p
Full model: species x treatment + body index, nested for individuals and brood						
Number of individuals: 34, from 18 broods						
displays	Treatment	0.22	0.40	0.31	1,31	0.58
	body condition	0.05	0.20	0.06	1,30	0.81
	brood					1.00
time	Treatment	0.03	0.21	0.01	1,31	0.90
	body condition	0.02	0.12	0.12	1,30	0.89
	brood					1.00

Table2 Results of the GLMM of the mate choice experiments.
Effects are listed in reverse order of removal from the model. In bold the final model, which only includes significant effects. The two tests for each individual were always retained in the model. When none of the fixed factors was significant (which was the case for both variables), the analysis shows whether the variable is significantly different from equal response to both intruders. The estimates of the proportion behaviour (or time) were in neither variable significantly different from 0.5, indicating no species specific preference.

males showed no difference in preference measured in the displays between the two trials F(1,33)=1.37, P=0.24, effect size: 0.05 +/- 0.04 SE, but spent more time with heterospecific females in the second trial F(1,33)=2.55, P=0.01, effect size 0.05 +/- 0.02. Figure 3b (for *P. nyererei*) and 3c (for *P. pundamilia*) show the change in the proportion of time spent with the conspecific female between the two trials.

Regression of Aggression and Mate Choice Behaviour

There was no significant relation in any combination between the degree of assortative courtship displays and assortative aggressive behaviour: LD-Q: F(2,30)=0.25, P=0.761, r^2=0.02; courtship displays and FD-B: F(2,30)=0.30, P = 0.722, r^2=0.02; courtship displays and time spent interacting with intruder: F(2,30)=1.34, P = 0.232, r^2=0.08.

There was also no significant correlation in any combination of the proportion of time a male spent with a conspecific female and assortative aggressive behaviour: LD-Q: F(2,30)=0.13, P = 0.871, r^2=0.01; FD-B: F(2,30)=0.33, P=0.705, r^2=0.02, time spent with conspecific female and time spent interacting with conspecific intruder: F(2,30)=0.16, P=0.844, r^2=0.01.

Discussion

With a cross-fostering experiment, we tested whether imprinting on the maternal phenotype mediates male mate preferences and aggression biases in two closely related species of Lake Victoria cichlids. Imprinting may yield

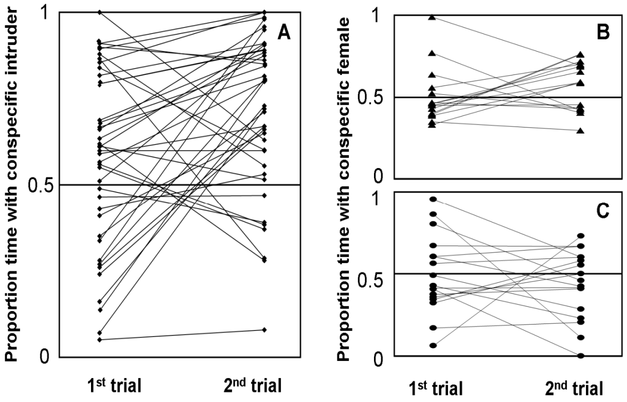

Figure 3 Differences between trials. The lines connect the two scores of an individual. **A**. Aggression tests of both species. Proportion of the time spent interacting with the conspecific intruder increased from trial 1 to trial 2. **B** *P. nyererei* mate choice trials. Proportion of the time spent with the conspecific female increased with trial 1 to trial 2. **C**.*P. pundamilia* mate choice trials. Proportion of the time spent with the conspecific female decreased slightly from trial 1 to trial 2.

strong assortative behaviour, which is critical for species coexistence and also for sympatric speciation. There was no evidence that males imprinted on their mothers' phenotype for their later aggression bias. Male aggression in territorial defence was directed primarily towards conspecific intruders, in males of both species and of both treatments. They showed therefore the same aggression bias as males showed in the wild, and wild caught males in laboratory experiments (Dijkstra et al. 2006b). However, we did find that the males' behaviour changed with experience, since they showed a stronger aggression bias to conspecific males in the second trial.

It is also highly unlikely that imprinting affects male mate preferences for conspecific females. An interaction with the females' preferences could obscure male mate preferences, if males stop displaying to females that are uninterested. However, there was no difference in response ratio between the two females in a mate choice test, and the response ratio of the females was quite high. Although there may be other, more subtle cues from the female that we were not able to measure, from our data it seems unlikely that males may have displayed against their preference due to the females' behaviour. We found some indication that *P. nyererei* males have a preference for conspecific females. Pierotti & Seehausen (2007) showed evidence for morph specific male mate preferences in a Lake Victoria cichlid species (*Neochromis omnicearuleus*) which shows three distinctly different morphs. It is therefore not unlikely that males of *P. nyererei* show preferences for females of their own species over heterospecific, congenic females. However, we point out that our sample sizes are quite low. While the overall effect of a lack of an effect of cross-fostering on mate choice seems quite robust, the data do not allow a more fine tuned analyses of biases or species differences, and any firm conclusions on these issues are at the moment premature. Finally, we found no relation between a bias in a male's territorial defence and his mate choice behaviour. We tested for this because a males' preference for conspecific females may have implications for its perception of territorial competitors, or vice versa, however our results provide no evidence for this. It can be difficult to test for such relationships, since this requires that individuals' experiences are controlled until the time of testing. Although the aim of this study was not to test for such a behavioural coupling, since the males had only had experience with other males until their first mate choice test, this study provided an opportunity.

The absence of an effect of cross-fostering in both male mate choice and male territorial defence contexts is in contrast with the finding that females do show sexual imprinting on their mother (chapter 3). Our conclusion is that the sexes show a difference in development for species recognition in sexual contexts. Such results bear some resemblance to those of Barlow (1992), who found that males of the Middle American Midas cichlid (*Cichlasoma citrinellum*) also show no signs of imprinting, however he found no strong effects of sexual imprinting in females. Sex differences in sexual imprinting have also been reported for several bird species (e.g ten Cate 1985; ten Cate & Vos 1999; Witte & Sawka 2003). Many of these may be explained by the use of different cues for partner selection by the two sexes (ten Cate 1985; ten Cate & Vos 1999). Preferences for such different cues (such as body size, colour pattern, colour intensity, behaviour, sounds etc.) may be affected in different ways by early development (ten Cate 1985). The apparent difference between the sexes in the cichlid species in this study may have a similar underlying explanation, because females and males of these species are sexually dimorph. For instance, females exhibit a much lower level of coloration compared to the males, and males and females also differ in other morphological aspects (Seehausen et al. 1998; chapter 3). Thus, males and females may, due to the different cues available to both sexes, use different cues for mate selection.

Although an absence of evidence for imprinting in the males in this study may not be evidence of absence of imprinting, a difference between the males and females for the effect of cross fostering may make sense in the light of skewed reproductive potential. The asymmetry in reproductive investment in these lekking species would predict that females are under strong selection to be choosy and males to be less discriminating (Trivers 1972). Female cichlids may therefore have endured stronger selection pressures to develop a mechanism for assortative mating. However, there is accumulating evidence for male mate preferences in lekking species (Saether et al. 2001; Werner & Lotem 2003; Werner & Lotem 2006; Pierotti & Seehausen 2007). For instance in lekking great snipe *(Gallinago media)*, males may forgo matings with a female he already mated with, in favour of a future opportunity (Saether et al. 2007). Such male mate preferences in lekking species indicate that males also have a limited reproductive potential, and therefore can be expected to show some choosiness. Though interspecific mating between *P. nyererei* and *P. pundamilia* produces viable and fertile offspring (Seehausen et al. 1997, van der Sluijs *in* *press*), such hybrid offspring may be less attractive and hence suffer from a decreased fitness. *Pundamilia nyererei* males may therefore forgo such mating opportunities in favour of species assortative matings.

If male territorial aggression biases and male mate preferences are not mediated by sexual imprinting, the question remains open as to what mechanism does mediate them. Male territorial aggression biases in these species can be influenced by experience, which has been shown in a study on the same species (Dijkstra et al. 2006a). Interestingly, we found an increase in assortative male-male aggression from the first trial to the second, also suggesting an influence of experience on male aggression (figure 3a). Also for the mating preferences we observed an effect of experience, at least in *P. nyererei*, which showed an increase in assortative mate preferences for *P. nyererei* females from the first trial to the second (figure 3b). In another study, males that had experience with males from both species showed more aggression towards their own species, while males only exposed to their own species showed no such bias (Dijkstra et al. 2006a). Such results suggest that experience with other species may be necessary for the development of an aggression bias. The results of our study, however, show that this need not be the case, because the males in our experiment, which were raised by a conspecific female, had never been in contact with males from the other species, yet they showed more aggression towards conspecifcs. Another type of experience that could have affected male behaviour is that males in this experiment may have learnt about their brothers' phenotype. The full broods were cross-fostered, and males were kept with their male siblings until the time of testing. In another study on a species pair of a different genus we show that such an effect can occur (chapter 5). Here we show that males do not show a difference in aggression bias as a result of experience with a foster mother of a different species. Alternatively, a predominantly genetic background for differences in territorial defence biases and mate preferences may also explain the observed behaviour, not withstanding the indication that adult experience may shape territorial defence behaviour in these cichlids. More experiments are needed in order to rule out these alternative hypotheses.

Both species showed clear species assortative behaviour in male-male interactions. The strong assortative character of male-male aggression may contribute to frequency dependent intra sexual selection, which is suggested to have contributed to the sympatric occurrence of haplochromine cichlids in general (Mikami et al. 2004; Seehausen & Schluter 2004) and for *P. pundamilia* and *P. nyererei* in particular (Dijkstra et al. 2005; 2006b; 2007). If male aggression biases are at least partly formed by adult experience, which seems to be the case (this study and Dijkstra et al. 2006a), then they can adjust, from one generation to the next, to a changing composition of species and phenotypes in the population. Such flexible aggression biases may be counter to a frequency dependent intra sexual selection scenario.

Strong male mate preferences for conspecific females would aid the development of assortative mating (Almeida & de Abreu 2003; van Doorn et al. 2004), and would thus make a scenario of sympatric speciation more likely. The species specific male mate preferences in *P. nyererei* may help this way. However, our findings give some indication that their preferences may be affected by courtship experience, in which case the male mate preferences could be indirectly shaped by those of the females. While the net result is species assortative mate preferences in both sexes, a developmental mechanism independent of encounters with unrelated females would probably promote reproductive isolation more straightforwardly. To conclude, although these results contribute to the understanding of how the species assortative behaviour may have arisen in these cichlids, the picture is still far from complete.

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Chapter 5

Females learn from mothers, and males learn from others. The effect of mother and siblings on the development of female mate preferences and male aggression biases in Lake Victoria cichlids, genus *Mbipia*.

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Abstract

While species assortative behaviour is often observed in sympatrically occurring species, there are few examples where we understand the extent to which development of assortative behaviour is genetically or environmentally determined, for instance through learning. However, the majority of mate choice theory assumes genetic recognition mechanisms. Knowledge about the development of species recognition is important for our understanding of how closely related species can coexist, and how this coexistence may have arisen. The ontogeny of female mate choice, for instance, may critically influence the degree of assortative mating under many circumstances. Also, male assortative aggression behaviour may affect fitness, and the possibility for coexistence of two closely related species. Here we test whether male aggression biases and female mate preferences of two Lake Victoria rock cichlid species, *Mbipia mbipi* and *Mbipia lutea*, are affected by experience. With an interspecific cross-fostering experiment, we test the effect of experience with the phenotype of the mother and that of the siblings on species assortative mate preferences and aggression biases. We demonstrate that female mate preferences are strongly influenced by learning about their mothers' phenotype, but not 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. We suggest that the development of assortative behaviour of females, but not of males, creates favourable conditions for sympatric speciation in Lake Victoria cichlids.

Key words: sexual imprinting, rival imprinting, speciation, East African cichlids.

Introduction

Stable coexistence of closely related species is possible with reproductive isolation to prevent hybridization and a source of negative frequency dependent selection to maintain both species (e.g. van Doorn et al. 2004; Rueffler et al. 2006; Dieckmann & Doebeli 1999). Reproductive isolation is mediated by preferences for conspecifics as potential mates. However, individuals of species that share their habitat with closely related species face the every day problem of deciding whom to interact with. How do individuals develop the selectivity to interact preferentially with conspecifics? Several developmental mechanisms can produce such assortative preferences, ranging from a fully genetically controlled development to fully environmentally determined preferences (Riebel 2003b; Shaw 2000). Some of these mechanisms, however, will not produce assortative behaviour in all circumstances, while other mechanisms may particularly easily produce species assortative interactions under many circumstances (Dieckmann & Doebeli 1999; Servedio 2000; van Doorn et al. 2004; Arnegard & Kondrashov 2004; Beltman & Metz 2005, this thesis chapter 2). The developmental mechanism behind preferences and biases in social interactions may therefore play a pivotal role in the evolution of species assortative behaviour and therefore speciation. The study of its development may help us understand these processes.

To keep one species from out competing the other species, or to prevent chance fluctuations in population size to eliminate one of the species, a source of negative frequency dependent selection is needed (reviews in Kirkpatrick & Ravigne 2002; Rueffler et al. 2006), which may maintain both phenotypes, and also drive the evolution of new species. Natural selection through competition over resources may be one such source. An alternative or an additional source for frequency dependent selection has been indicated in male-male competition (Seehausen & Schluter 2004; Mikami et al. 2004; van Doorn et al. 2004). Males may be better off by selectively fighting only with males that compete for the same females or resources, and assortative male-male aggression would be adaptive in many cases. Male aggressive behaviour could therefore also exert selection on species specific phenotypes (e.g. Alatalo et al. 1994; Seehausen & Schluter 2004; Tynkkynen et al. 2005). 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 (Seehausen & Schluter 2004). The reduced male-male competition for rare phenotypes should then result in increased mating opportunities. Speculating on how this may be achieved; males may be able to invest more in courtship, or may be preferred by females, for instance because they are in better condition after less fighting (Wong & Candolin 2005). A rare-male advantage arises from strictly assortative male-male interactions, thus proximately mediated by the development of selective male aggression. 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.

Although the occurrence of species assortative behaviour is relatively common, in comparison only a few studies have looked at what developmental mechanism mediates it, and often only in one of the sexes (fish: Engeszer et al. 2004; this thesis chapter 3; songbirds: ten Cate & Vos 1999; Slagsvold et al. 2002; Hansen & Slagsvold 2003; Riebel 2003b; corn borer moths: Roelofs et al 1987; crickets: Shaw 2000; Ritchie 2000; fruit flies: overview in Coyne & Orr 2004). As a consequence, our understanding of the possible evolutionary trajectories leading to assortative behaviour and reproductive isolation is limited. One developmental mechanism mediating mate preferences that is found in several species is early learning by young about a parental phenotype as a model for their later preferences for mates (Kendrick et al. 1998; ten Cate & Vos 1999; Bereczkei et al. 2004, this thesis chapter 3). This mechanism, also known as sexual imprinting, matches an individual's preference to the parental phenotype. High genetic, and therefore phenotypic, similarity between parent and offspring appearance will ensure a relatively good match of social preferences and own phenotype. While often studied in the context of mate preferences, male-male interactions may be mediated by imprinting also, called rival imprinting (Vos 1994; Hansen & Slagsvold 2003). Alternatively, a genetic predisposition for 'own type' bias may also provide assortative social interaction patterns. In contrast, influence from encounters with less related individuals would clearly not produce assortative behaviour. In many animal species this includes virtually all learning after independence, due to dispersal from their natal area.

A particular group of species where coexistence of closely related species occurs regularly are the haplochromine rock cichlids from East Africa, with about 1100 species in the three great lakes (Seehausen 2000; Turner et al. 2001). They are especially interesting to study assortative behaviour because this seems to mediate their reproductive isolation. The large number of species suggests that haplochromine cichlids have properties that make them coexist with many closely related species (Salzburger et al. 2005; Seehausen 2006a). Moreover, the ecological differentiation between sympatric species pairs seems limited (Seehausen & Bouton 1997; Genner et al. 1999a), thereby minimizing the role for ecological competition in frequency dependent selection. In the laboratory, haplochromine cichlid species can interbreed, and hybrid infertility has not yet been observed (Van der Sluijs et al. *in press.*; Seehausen 2004). Thus, haplochromine cichlids do not appear to have built up genetic incompatibilities between species which would prevent them from interbreeding, suggesting that reproductive isolation is almost entirely mediated by mate choice. It has been hypothesized that male-male interactions yield negative frequency dependent selection in cichlids (Seehausen & Schluter 2004). In males, territorial ownership is a requirement for mating, and males will defend their territories vigorously against competing males. Such territories are in general for mating purposes only and are not or very rarely used for feeding (Seehausen & Schluter 2004). Also, males more often have neighbouring territory owners of another species than their own species, a pattern predicted when male-male aggression indeed yields negative frequency dependent selection (Seehausen & Schluter 2004). Moreover, male-male aggression is biased towards males that are likely to compete for the same females (Lake Victoria: Dijkstra et al. 2006b; Dijkstra et al. 2007; Lake Malawi: Genner et al. 1999b).

How might assortative behaviour in these cichlids be achieved? The exclusive and prolonged maternal care in haplochromine cichlids provides the young with the opportunity for learning about their mothers' phenotype. Such learning may produce assortative social preferences. We showed in an interspecific cross-fostering experiment that females of a closely related species pair (species *Pundamilia pundamilia* and *P. nyererei*) from Lake Victoria indeed imprint on their mothers' phenotype (chapter 3). However, the males from the same cross-fostering experiment did not show any difference in their aggression bias when fostered by a female of a sister species or of their own species; all males selectively direct their aggression to conspecific males (chapter 4). However, there are indications that experience with conspecifics at a later age may affect the direction of male aggression (Dijkstra et al. 2006a, this thesis chapter 4). There is some indication from a study on *Astotilapia burtoni* that young individuals may learn from their siblings (Crapon de Caprona 1982). Since in our earlier cross-fostering experiment males had exposure to their male siblings, they also may have learnt about their phenotypic characteristics. The females in that experiment, on the other hand, were not allowed interaction with males from the age of onset of sexual dimorphism onwards. However, interactions among sub adults may occur frequently in nature, and such experience could potentially diminish the consequences of imprinting for assortative mating.

In this paper we address the relative influence of experience with the maternal phenotype and the phenotypes of siblings on female mate choice and male rival recognition. We present a cross-fostering study in the closely related, ecologically similar and sympatrically occurring species from Lake Victoria: *Mbipia mbipi* and *M. lutea*. They show overlap in morphology, feeding ecology and time and place of breeding, while male nuptial coloration is distinctly different, which is typical for such closely related species pairs (Seehausen et al. 1998). We obtained cross-fostered individuals by swapping full broods between brooding females, as well as swapping almost the full brood or only a few eggs. This way we test the effect of both learning from mothers' phenotype as well as learning from their siblings' phenotype. By allowing the broods to grow up as either pure species groups or as mixed species groups we mimicked possible skewed social experience individuals may encounter while maturing. We tested females for their mate preference and males for their aggression bias. In this study we did not test for male mate preferences, although they would be interesting to address in future studies.

Methods

Reproductive behaviour

Adult males will defend territories in order to attract females. The various aggressive and courtship displays of males and females are extensively described in Baerends & Baerends-van Rhoon (1950). Territories in these limnetic species are only for reproductive means, feeding occurs outside the territories (Seehausen & Schluter 2004). Aggressive interactions usually will take place at the border of the territories, to repel intruders. There are two categories of behavioural display that we scored during observations of the aggressive interaction observations . The first category is formed by attack-like displays, called frontal displays, which precedes the biting of each others mouth, used at the border of a territory. Both these behaviours (frontal displays and biting) were taken together in one frontal behaviour measure by adding up the instances of each behaviour. The other category is formed by lateral displays, which may be followed by quivers. These behavioural elements are often used in within-territory fights. Like for the frontal behaviour, we took these two displays in one lateral display measure.

Courtship interaction often starts at the boundary of a territory, and consists of a sequence of displays. During courtship the male will approach a female when she enters the territory and the male will show a lateral display, followed by a quiver and a lead swim towards the center of his territory. The female can respond to these behaviours by approaching the male and by following his lead swim. The male will lead the female to the center of its territory, where spawning may take place. We scored the type of displays a male gave in a mate choice test, and the subsequent approach behaviour of the female.

Raising of the treatment clutches

We raised clutches in 4 different treatment groups, in which mother, siblings or both could be either a conspecific or heterospecific of the focal individual. We refer to them by abbreviations: the first letter indicates a con- or heterospecific mother, the second letter indicates the siblings: $C_{mo}C_{sib}$: conspecific mother, conspecific siblings; $C_{mo}H_{sib}$: conspecific mother, heterospecific siblings; $H_{mo}C_{sib}$: heterospecific mother, conspecific siblings and $H_{mo}H_{sib}$: heterospecific mother and heterospecific siblings (Figs. 1 and 2).

Each of the four treatments consisted of exchanging eggs between brooding females. Broods contained on average 22.6 eggs, minimal 15, maximal 41. In the $C_{mo}C_{sib}$ treatment we exchanged the full clutch between two females of the same species. In the $C_{mo}H_{sib}$ group we exchanged all but 3 to 6 focal eggs between two females of the different species. In the $H_{mo}C_{sib}$ group we exchanged full clutches between females of the different species and in the $H_{mo}H_{sib}$ group we exchanged 3 to 6 focal eggs, between two females of the different species. Mouth brooding takes 3-4 weeks, then the female releases the fry from her mouth. Fry start foraging independently from that moment on. Females of these species display fry guarding behavior during up to 3 weeks. The brooding females were allowed to show their normal brood care behaviour. After the extended brood care period, the foster mother was removed from the experimental clutch and returned to the stock tank. The clutch was moved to a larger tank when they were between two to three months old, and remained there until they were sexually mature (approximately 8 months). In nature sibling groups may not stay together this long, but we decided for this treatment to maximize any possible effect of experience with siblings. After both sexes had fully developed, as judged from their nuptial coloration, but before any breeding had occurred in the tank, the focal females and males were removed from the tank and isolated approximately two weeks before testing.

We were able to raise 4 $C_{mo}C_{sib}$ clutches, two of each species; 4 $C_{mo}H_{sib}$ clutches, only *M.lutea*; 7 $H_{mo}C_{sib}$ clutches, 2 *M.mbipi* and 5 *M.lutea*; and 6 $H_{mo}H_{sib}$ clutches, 3 of each species. Because the $C_{mo}H_{sib}$ and $H_{mo}H_{sib}$ groups each yielded only a few individuals per clutch (due to the design of the treatment, average ratio of individuals of the focal species to heterospecific individuals was 1:8), and a few clutches contained either experimental males or females, our sample size was too small to analyze the data on a per species and per treatment basis. We therefore merged the data of both species in this experiment.

Female mate choice tests

We performed 38 successful preference tests on 20 females (4 $C_{mo}C_{sib}$, 3 $C_{mo}H_{sib}$, 5 $H_{mo}C_{sib}$, 8 $H_{mo}H_{sib}$), from in total 13 broods. Each female was tested twice, except for two females, who did not become gravid again after their first trial. The test setup and the scoring method was the same as described in chapter 3. The experimental tank (2 x 0.5 x 0.5m) was divided into 3 equal compartments by two grids with mesh-size 160 x 160 mm. In the outer compartments a *M. mbipi* and *M. lutea* male were placed and bricks were provided as territorial ‘rocks’. The mesh size prevented the males from going through the grid, but the females were smaller than the males and were able to swim freely through the tank. In the middle compartment shelter for the female, in the form of a PVC tube, was provided. Males were matched for standard length as much as possible (average standard length difference was 0.6 mm +/- 0.09 SE, average 0.75 % difference relative to the largest male). Males were placed into the experimental tank one day before testing. Twenty stimulus males of each species were (re)combined to 22 stimulus pairs. The average number of times each stimulus pair was used was 1.73 +/- 0.18 SE. A gravid female (i.e ready to lay eggs, as judged by the swelling of the abdomen) was placed in the middle compartment 30-60 minutes prior to testing while opaque sheets hid the males from her. Then the sheets were removed, and the female could reach and see the whole tank. A trial lasted 30 minutes. Courtship behavior was scored as described in Seehausen and van Alphen 1998 and this thesis chapter 3. In a successful trial both males displayed at least two quivers and the female responded positively to a quiver twice. If these criteria were not met, testing was later repeated. We tested until each female had two successful trials, with different male pairs and with species and tank side fully counterbalanced. Preference scores used in statistical analysis are the approach ratio to the displays of the conspecific male minus that to the heterospecific male. For instance for quiver displays: (# approaches to conspecific male / # quiver displays conspecific male) – (# approaches to heterospecific male / # quiver displays heterospecific male). The resulting scores were therefore on a scale between 1 and -1, which we used as a measure for preference for conspecific males and heterospecific males respectively.

Male aggression bias tests

We tested 11 $C_{mo}C_{sib}$ males, 4 $C_{mo}H_{sib}$ males, 8 $H_{mo}C_{sib}$ males and 8 $H_{mo}H_{sib}$ males, from a total of 19 broods. Each male was tested twice, except for two males, one in the $H_{mo}C_{sib}$ group and one in the $C_{mo}H_{sib}$ group, who died before the end of the experiment. The focal male was placed 48 hours prior to testing in the experimental tank (2 x 0.5 x 0.5m), which was divided in half with the use of 2 opaque PVC sheets. Each male also had a 'neighbour male': a smaller male cichlid of a different genus placed at the end of the tank behind a Plexiglas sheet. We placed this male in the experimental tank to enhance territoriality (Dijkstra et al. 2006b). At the time of testing, two cylinders were placed in the experimental space of the focal male. In these cylinders we placed a male of each species, matched for standard length to each other (mean difference in standard length was 0.4 mm +/- 0.06 SE, average 0.8 % difference relative to the largest male). Thirty two *M. mbipi* and 34 *M. lutea* males were (re)combined to 44 stimuli pairs. The average number of times each stimulus pair was used was 1.59 +/- 0.13 SE. Observations started right after placement of the cylinders, and lasted 20 minutes after the start of aggressive interactions. Behaviours scored were: frontal displays, bites, lateral displays and quivers. Aggression bias as analysed in the statistical analysis was the proportion behaviour directed at the conspecific male, which therefore yields a score between 0 and 1, with a score above 0.5 indicating an aggression bias to males of their own species.

Statistical analysis

All statistical analyses were conducted using R statistical software (R Development Core Team 2005). We fitted Generalised Linear Mixed Effect Models (GLMMs). All models were hierarchically nested, correcting for any pseudoreplication: broods within treatment, and individuals (two trials) within broods. We had two fixed effects which each had two levels: foster mother (conspecific or heterospecific) and siblings (conspecific or heterospecific). If the fixed effect of foster mother is significant in the model, the behaviour of the males or females is affected by the phenotype of the (foster) mother that raised them. If the fixed effect of siblings is significant in the model, the behaviour of the males or females is influenced by the phenotypes of their (foster) siblings. An interaction would indicate that each of the treatments had a different effect in each combination. We stepwise deleted factors from a fully saturated model until the minimal adequate GLMM was found, but always keeping repeated measures for each individual as a random factor. Depending on the nature of the data, we used the Gaussian (standard normal) or the binomial distribution to fit our models. For data analysed with Gaussian distribution we report F-tests on the variance, while we report a X^2 test on the deviance, as appropriate for binomial data.

At each step in the model simplification, we verified that the assumptions of normal distribution of the errors and heteroscedastity were met, by visual inspection of the concerned plots, and for overdispersion when the binomial distribution was used. We did not test for significant female mate preferences or male aggression biases per treatment group, due to the limited sample size in each separate group.

Results

Although we cannot statistically test for species differences, inspection of the data obtained from the two types of tests in males and females showed that *M.mbipi* fell safely within the range of *M.lutea*, for which we obtained more data.

Female mate choice tests

We found a significant effect of the species of the (foster) mother on the mate choice behaviour of the females (figure 1). Females were more likely to

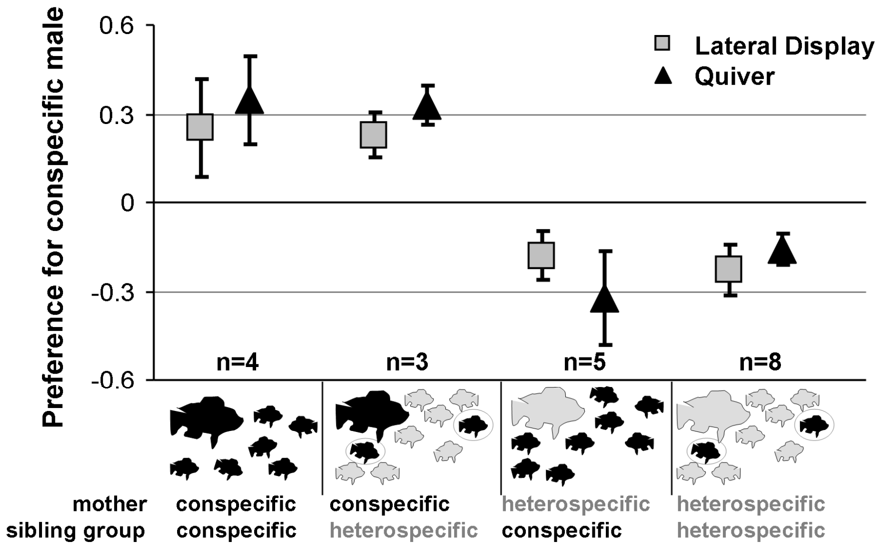

Figure 1 Female preferences in the different treatment groups. Grey squares are lateral displays, black triangles are quivers,; means +/- SE. Scores above zero reflect a preference for conspecific males, scores below zero reflect a preference for heterospecific males.

Response variable	Explanatory variable		df	*P*
Lateral Display	distribution: Gaussian	*F*		
	Fixed effects:			
	mother	18.11	1,18	<0.001
	siblings	0.34	1,17	0.57
	mother x siblings	0.01	1,16	0.94
	Random effect:			
	Individual	Lh. ratio = 0.1301		0.717
	brood	Lh. ratio<.0001	1	
Quiver Display	distribution: Gaussian	*F*		
	Fixed effects:			
	mother	29.63	1,18	<0.001
	siblings	0.80	1,17	0.38
	mother x siblings	0.005	1,16	0.94
	Random effect:			
	Individual	Lh. ratio < 0.0001		1
	brood	Lh. ratio<.0001	1	
First visit	distribution: binomial	$\mathbf{X^2}$		
	Fixed effects:			
	mother	6.32	1	0.01
	siblings	0.15	1	0.67
	mother x siblings	0.02	1	0.99
	Random effect:			
	Individual	0.1677	1	0.6821
	brood	0	1	1
Time spent	distribution: binomial	$\mathbf{X^2}$		
	Fixed effects:			
	mother	2.81	1	0.09
	siblings	1.02	1	0.31
	mother x siblings	0.06	1	0.81
	Random effect:			
	Individual	<0.001	1	0.999
	brood	0	1	1

Table 1 Results of the GLMM's of analysis of the female preference tests. The effects are listed in reverse order of deletion from the model. The final model is in *italic*.

approach a displaying male if he was of the same species as their foster mother. This was true on both the level of lateral displays (*F*(1,18)= 18.11, *P*<0.001) and on the quiver displays (*F*(1,18)= 29.63, *P*<0.001). Females showed a trend to spending more time with the male of the same species of their foster mother (X^2=2.81, df=1, *P*=0.09). There was no effect of siblings in either measure (figure 1, table 1).

We tested for an effect of treatment on which male a female visited first in a trial. This first visit was more often than random to the male of the foster species (X^2=6.32, df=1, *P*=0.012) (table1). Again, siblings had no effect on the choice of first visit. Additionally, we tested if the male a female visited first in a trial was predictive of her approach rate to either lateral display or quiver display. There was a significant relation between first visit and differential approach rate to the quiver display (X^2=5.7, df=1, *P*=0.017), and a trend with lateral display (X^2=2.75, df=1, *P*=0.09).

Male aggression bias tests

We found an effect of sibling group, but not mother, on the direction of aggression by the males (figure 2) This was significant in all three parameters: the proportion frontal behaviour directed to the conspecific intruder (X^2=13.17, df=1, *P*<0.001), the proportion lateral behaviour (X^2=17.59, df=1, *P*<0.001) and the proportion of time spent interacting with the conspecific intruder (X^2=10.77, df=1, *P*=0.001). Males with conspecific siblings showed more displays towards and spent more time interacting with the conspecific intruder, than males raised with heterospecific siblings. Males displayed more often with frontal behaviour than with lateral behaviour (paired t test: t_{36} = 5.39, *P*< 0.001, mean of the differences: 48.2). The random effect 'individual' was significant in both the frontal behaviour and lateral behaviour, we therefore subsequently tested if this was due to a difference in the behaviour between the first trial and the second trial. We also tested if such a 'trial effect' was

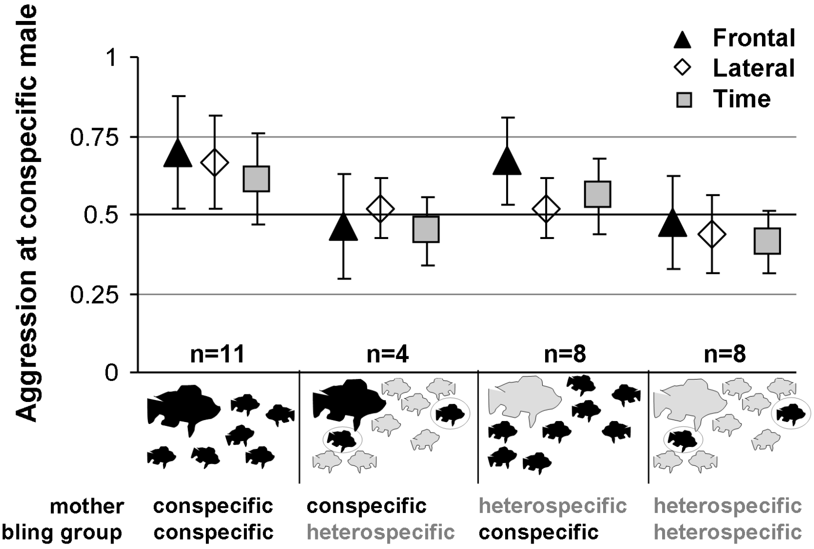

Figure 2 Male aggression direction of the different treatment groups. Black triangles are frontal behaviour, open diamonds are lateral behaviour , grey squares are time spent interacting; means +/- SE. Scores above 0.5 reflect more aggression to conspecific intruders, scores below 0.5 reflect more aggression to heterospecific intruders.

Response variable	Explanatory variable	X^2	df	*P*
Frontal behaviour	Fixed effects:			
	siblings	13.17	1	<0.001
	mother	0.07	1	0.80
	mother x siblings	0.0003	1	0.99
	Random effect:			
	Individual	8.91	1	0.0028
	brood	0	1	1
Lateral behaviour	Fixed effects			
	siblings	17.59	1	<0.001
	mother	0	1	1
	mother x siblings	0	1	1
	Random effect:			
	Individual	32.41	1	<0.001
	brood	0	1	1
Time spent	Fixed effects:			
	siblings	10.767	1	0.001
	mother	1.5044	1	0.22
	mother x siblings	0.076	1	0.78
	Random effect:			
	Individual	0	1	1
	brood	0	1	1
Effect of trial:				
FD-B	trial	2.06	1	0.11
	siblings	15.52	1	<0.001
	trial x siblings	0.01	1	0.94
LDQ	trial	0.15	1	0.67
	siblings	7.95	1	0.004
	trial x siblings	0.01	1	0.90

Table 2 Results of the GLMM's of analysis of the male aggression tests. The effects are listed in reverse order of deletion from the model. The final model is in *Italic*. The final models testing for the effect of trial also show non significant interaction effects, but the deletion of the interaction effect did not make the trial effect significant. We therefore kept the full model as final, showing no effects of trial

different between the treatments. We did not find such effects (table 2).

Discussion

Our data show effects of experience on both female choice and male-male aggression in these cichlid species. The females raised in our experiment developed a sexual preference for males of their mothers' phenotype. We tested females when they were sexually mature, at which time their (foster) siblings were also fully developed and their brothers thus showed nuptial coloration. In two of the four treatment groups: $C_{mo}H_{sib}$ and $H_{mo}H_{sib}$, females therefore had the opportunity to interact with males of a different species than that of their foster mother, but this caused no shift in preference towards the species of their siblings. So, despite ample opportunity for interaction with their (foster) siblings, this did not affect their mate preference. Our results thus show that imprinted preferences can be robust against later social experiences. In a previous cross-fostering study on another species pair, females of *P. pundamilia* and *P. nyererei* showed the same effect of foster mother on their mate preferences (chapter 3). The high similarity between the results of both studies supports the idea that the closely related haplochromine cichlid species of Lake Victoria share this imprinting behaviour. Because females imprint on their mothers' phenotype, rather than on other individuals, there is a strong link between the phenotype of the female and her preference for males with a similar phenotype (chapter 2). This is a condition for strong assortative mating patterns, critical for both a sympatric speciation scenario and reinforcement after secondary contact (e.g. Felsenstein 1981; Servedio 2000).

In contrast to the females, males adjusted the direction of their territorial defence depending on the composition of the brood they grew up in. Males showed more aggression to heterospecific males if they had been raised with heterospecific sibling males, while the species of foster mother had no significant effect on the parameters we measured. The males' aggression thus was not directed at their own phenotype at all times, but instead at males similar to the males they were raised with. Under normal conditions (i.e. not when cross fostered) siblings would resemble a males' phenotype more than in our mixed broods cross fostering. However, offspring shares on average more of the genotype, and phenotype, with their mother than with their siblings. Even though some aspects of the female's phenotype are different from the male's phenotype, there are apparently cues available to generalise from the mother's phenotype to the male's phenotype as shown by the females in this study and in chapter 3. Rival imprinting on the mothers' phenotype would thus more likely lead to species assortative aggression than when males imprint on their siblings.

Species differences in the effects of imprinting could not be tested for with the data obtained in this study. Although we did not find any species differences in our previous cross fostering study, a difference in early learning between closely related species was shown in studies on birds (Slagsvold et al. 2002; Hansen & Slagsvold 2003).

Sexually mature males are brightly colored, while females are mostly yellow or brown-grey. However, there is some difference in the coloration of females between the two species, and this difference is also enhanced during mouth brooding (Seehausen et al. 1998). The young females may thus possibly have learnt about their mothers' colour, but it is equally possible that olfactory cues have been imprinted on. As both colour and chemical cues could be used at all stages of the experiment, we cannot conclude which cue served as imprinting stimulus.

While we can conclude that females learnt about their mother in the first few weeks of their lives, either during mouth brooding or while the mother guards the fry, when the males learnt cannot be assessed from this experiment.

If under natural conditions males learnt while hatching, or shortly thereafter, they are likely to imprint on closely related individuals, because the other hatchlings are at least half-siblings. But although they shared the time in the mouth of their (foster) mother with their foster siblings, it is not very likely that they learnt about them at that time. During this stage, there are no discernable visual differences between fry (Fernald & Hirata 1979), and the available light in the mouth of the mother is likely to be quite limited. This implies that if the fry learn about their siblings at this stage, they probably would have to rely on olfactory cues. However, the olfactory cues in the mothers' mouth are also likely to be largely influenced by the mother herself. Since we found no influence of species of foster mother on the males' behaviour, it seems unlikely that males use this experience while hatching. At a later stage, the females of these cichlids also provide care for their offspring after hatching by taking them back into their mouth with approaching danger (another fish, or in our experiment, an animal care taker). Fry then rely on visual cues, in order to approach the mouth of the female (Baerends 1993; Russock 1999). The interaction with their siblings at this time may provide them with the opportunity to learn about their fellow siblings, which would ensure that they learn about closely related individuals. While in our experiment the brood stayed in one tank exclusively, in nature it is largely unknown how and if young cichlids aggregate from the time their mother leaves them alone until the time they become territorial. However, schools of same size fry can often be seen in shallow water at the rocky shores (pers obs MNV), and mixed species shoals of non-breeding males have been observed (Seehausen et al. 1998 & pers com M.E. Maan). If males establish their aggression biases at that stage, those biases are not likely to be assortative, because the individuals they encounter at that time are likely to be from different species. However, this scenario would not explain the species assortative behaviour found in wild males of several reproductively isolated populations (Dijkstra et al. 2006b; Dijkstra et al. 2007). Interestingly, interactions with males of a different species do not necessarily result in a lack of an aggression bias for their own phenotype, and may even bias aggression towards their own phenotype (Dijkstra et al. 2006a). This suggests that the development of aggression biases is sensitive to the context of the interactions between males, and that males do not learn the same way from their encounters with various males. However this is at the moment speculative and more experiments are needed.

Lake Victoria rock cichlids have been proposed as a possible case of sympatric speciation. This was postulated on the basis of distribution patterns of many species pairs, where one of the two species is nested within the distribution of the other (Seehausen & van Alphen 1999). The species pair in this study also shows such nested distribution (Seehausen et al. 1998). Conditions allowing sympatric speciation are quite strict, calling for strong assortative mating mediating reproductive isolation at a very early stage of divergence, and a source of negative frequency dependent selection driving the divergence. The alternative scenario leading to the observed distribution pattern of related species is secondary contact after allopatric divergence (Bouton 2000). This still requires reproductive isolation, but frequency dependent selection does not need to drive the divergence. While the imprinting behaviour of the females could cause strong assortative mating patterns in both scenarios of speciation (Servedio et al. in press and this thesis chapter 2), the male-male interactions are not assortative at all times. The cross-fostering experiment may be an unlikely situation in nature; however at an incipient speciation stage hybridization may be more common, and mixed broods resembling our experiment can easily occur. We therefore propose that the developmental mechanism for male aggressive behaviour is unlikely to produce enough frequency dependent selection at the earlier stages of speciation to help drive sympatric speciation, although it needn't hamper speciation either.

In summary, the results of our experiment indicate that females of the species pair *M.mbipi* and *M.lutea* sexually imprint on their mothers' phenotype, while the males do not. Male behaviour was affected by experience with siblings. This is consistent with the findings in a previous cross-fostering study with the other species pair *P. pundamilia* and *P. nyererei* (chapter 3 and chapter 4). The similarity of the results indicates that the developmental mechanisms of assortative behaviour may be shared between the highly related cichlid species of Lake Victoria. Secondly, the development of assortative male-male aggressive behaviour is partly mediated by experience with other individuals than their mother, possibly their siblings in nature. Learning about siblings would provide males in reproductively isolated species with an aggression bias for their own phenotype, but in hybridizing populations this may most likely result in a lack of an aggression bias. The development of assortative behaviour of females, but not of males, therefore, creates favourable conditions for sympatric speciation. However, in a scenario involving secondary contact after allopatric divergence the behaviour of both sexes contributes to species coexistence.

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Fishing for rock cichlids.
Mhoja Kayeba (left) and three young fishermen on Makobe Island.

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Nederlandse samenvatting

Deze samenvatting is een verkorte en bewerkte versie van de algemene inleiding en samenvatting in het Engels, in hoofstuk 1, van dit proefschrift. Dit hoofdstuk bevat geen verwijzingen naar wetenschappenlijke literatuur, zie hiervoor de Engelse versie.

Haplochromine cichliden zijn muilbroedende vissen in de Oost Afrikaanse meren en rivieren, en vormen de talrijke en diverse bewoners van het Malawimeer, Tanganyikameer en Victoriameer. In de grote meren van Oost Afrika komen in totaal waarschijnlijk meer dan 1100 soorten cichliden voor. Het is een ecologisch zeer diverse groep van soorten, en vertoont daarbij ook een grote verscheidenheid aan heldere kleurpatronen, welke ze populair maakt bij aquarium liefhebbers. Elke soort komt telkens slechts in één van de meren voor, nergens anders, en de soorten binnen één meer zijn ook meer aan elkaar verwant dan aan de vissen uit andere meren. Dit betekent dat alle vissen uit één meer één gezamenlijke vooroudersoort hebben. De combinatie van de grote hoeveelheid soorten die ze gevormd hebben en de grote diversiteit die ze vertonen maakt cichliden een goed model systeem voor de studie naar soortsvorming.

Wat is soortvorming?

Soortvorming is kortweg het ontstaan van nieuwe soorten door de opsplitsing van een vooroudersoort. De nieuw ontstane soorten zijn zo van elkaar veranderd dat ze niet meer met elkaar paren. We spreken dan van reproductieve isolatie. Een van de grote vragen op het gebied van soortvorming is hoe deze reproductieve isolatie kan ontstaan. Tijdens soortvorming veranderen populaties van 1 soort van elkaar (ze divergeren), en paringen vinden plaats binnen elke populatie. Dit kan gebeuren doordat populaties geografisch van elkaar gescheiden worden, door bijvoorbeeld een berg, woestijn of een oceaan. Toevalsprocessen of gerichte selectie kunnen er toe leiden dat de populaties van elkaar kunnen gaan verschillen. Het is eenvoudig in te zien dat er dan reproductieve isolatie ontstaat, omdat het onmogelijk is voor individuen om een partner uit de andere populatie tegen te komen, laat staan mee te paren. Echter, wanneer de gedivergeerde populaties niet door een geografische barrière gescheiden zijn, zoals in het geval van de cichliden die in hetzelfde meer voorkomen, of als na een periode van geografische scheiding hun verspreidingsgebied weer uitbreidt zodat er overlap ontstaat, zal reproductieve isolatie niet meer vanzelfsprekend zijn. Het komt er dan op aan om selectief te zijn en actief een partner van de eigen soort te kiezen. Met andere woorden, er ontstaat een evolutionaire selectiedruk op de correcte herkenning van de eigen soort. De voorspelling is dat dit herkenningsmechanisme meer specifiek wordt, doordat er negatieve consequenties zijn aan het paren met individuen van de andere soort en het krijgen van hybride nakomelingen. Dieren met een voorkeur voor het paren met partners die lijken op henzelf zullen zich dan succesvoller voortplanten, omdat ze geen hybride nakomelingen krijgen. De voorwaarden voor soortvorming zijn dus dat zowel de fenotypes[1] als de partner voorkeuren uit de populaties van elkaar veranderen. Bovendien zullen deze veranderingen ook als het ware in elk individu gekoppeld moeten zijn, omdat voorkeur en fenotype anders mismatchen en er alsnog hybride nakomelingen komen. Koppelen van twee kenmerken in het genoom is moeilijk, doordat recombinatie tijdens de meiose[2] meestal combinaties van genen breekt. De combinaties van genen zijn dus meestal niet overerfbaar. De koppeling van genen wordt moeilijker naarmate er meer genen bij betrokken zijn. De enige manier waarop morfologische eigenschappen en een genetisch bepaalde seksuele voorkeur hiervoor gekoppeld kunnen worden is doordat individuen paren met partners die dezelfde kenmerken hebben als zijzelf. Bij soorten die reproductief geïsoleerd zijn, worden dus slechts individuen van hun eigen soort herkend als potentiële seksuele partners.

De modelsoorten

In dit proefschrift beschrijf ik mijn onderzoek naar de invloed van vroege ervaring op het paargedrag en het territoriale gedrag van cichliden uit het Victoriameer. De centrale vraag hierbij is of deze ervaring op jonge leeftijd bijdraagt aan de ontwikkeling van soortherkenning. De modelsoorten die ik voor dit proefschrift gebruikt heb zijn twee paren van nauw verwante soorten. Het eerste paar bestaat uit de soorten *Pundamilia pundamilia* en *Pundamilia nyererei;* het tweede paar uit de soorten *Mbipia mbipi* en *Mbipia lutea*. Een kleurenfoto van elke soort staat op de omslag van de kaft. De soorten in elk paar overlappen in hun leefgebied, en bij beide paren vertonen de twee soorten grote gelijkenis in broedgedrag. Hoewel de cichliden in de Oost Afrikaanse meren een grote diversiteit in ecologie vertonen, vertonen de twee soortenparen een grote overlap in voedsel en habitat keuze. Zij verschillen wel in de kleuren van het broedkleed van het mannetje: *P.pundamilia* is blauw en *P.nyererei* is rood; *M.mbipi* is zwart en *M.lutea* is geel. Ze verschillen ook een beetje in hoe diep ze in het water voorkomen. Ze kunnen elkaar echter nog makkelijk tegenkomen, en ze zijn vaak op dezelfde plekken gezien tijdens duik observaties en gevangen in dezelfde netten. Ze zouden dus met elkaar kunnen paren, maar dit doen ze niet, en zijn ze dus reproductief geïsoleerd. De populaties die ik gebruikt heb voor dit proefschrift zijn afkomstig van het eiland Makobe, een rotseiland dat vijf kilometer uit de kust van de Tanzaniaanse kant van het Victoriameer ligt. *P. pundamilia* en *P. nyererei* zijn de modelsoorten voor veel van de studies naar partnerkeuze en soortvorming bij cichliden in het Victoriameer. Eerdere studies hebben aangetoond dat de vrouwtjes van deze twee soorten de mannetjes van hun eigen soort verkiezen boven die van de andere soort wanneer ze de keuze krijgen, en dat deze voorkeur deels bepaald wordt door de kleurverschillen in het broedkleed van de mannetjes. Binnen een soort paren vrouwtjes van *P. nyererei* het liefst met mannetjes die het felst gekleurd zijn, en die mannetjes blijken ook in betere conditie te zijn. Deze resultaten ondersteunen de hypothese dat de partnerkeuze van het vrouwtje de evolutie van de kleuren van het broedkleed van het mannetje heeft bepaald, maar laat de vraag open hoe vrouwtjes hun voorkeur ontwikkelen, en of de partner keuze ook reproductieve isolatie tot stand kan brengen.

Gedrag en evolutie

Gedrag kan bestudeerd kan worden om vragen op verschillende niveaus te beantwoorden. Men kan onderzoeken hoe gedrag zich in een dier ontwikkelt en hoe het fysiologisch georganiseerd is. Gedrag kan ook, net als alle andere eigenschappen van een dier, een evolutionaire aanpassing zijn, en evolueren, en dus leiden tot verschillen tussen soorten. Gedrag is in dat opzicht dus niet anders dan andere bijvoorbeeld morfologische kenmerken van een dier. Gedrag is echter wel speciaal omdat het direct ingrijpt op hoe individuen met elkaar omgaan, en het is deze interactie die ervoor zorgt dat ze hun territoria kunnen verdedigen, paren, en hun jongen kunnen opvoeden. Gedrag is dus de sleutel tot succesvolle voortplanting, en kan daarmee sterke invloed hebben op evolutionaire processen.

Soortherkenning is een belangrijke voorwaarde voor soortsvorming, en als we begrijpen hoe cichliden hun eigen soort herkennen, kunnen we ook meer begrijpen over hun evolutionaire geschiedenis. Deze vraag kunnen we eigenlijk splitsen in twee vragen, ten eerste: welke kenmerken gebruiken cichliden om hun eigen soort te herkennen (kleur, vorm van het lichaam, de geur). Deze vraag is voor diverse soorten cichliden al onderzocht, en daaruit blijkt dat zowel kleur, geur, als lichaamsgrootte belangrijk zijn. De tweede vraag die gesteld kan worden, is hoe soortherkenning zich ontwikkeld in een individu: leert het van zijn omgeving, van zijn ervaring met andere individuen of is het merendeels door de genen bepaald? Dit proefschrift behandelt mijn studie naar de ontwikkeling van soortherkenning bij cichliden uit het Victoriameer, en ik plaats deze in de context van hun soortvorming.

Wat is inprenting?

Soortherkenning kan zich ontwikkelen door inprenten. Konrad Lorenz, een van de grondleggers van gedragsbiologie, heeft het fenomeen inprenten beroemd gemaakt door als eerste duidelijk te laten zien dat ganzenkuikens leren over de kenmerken van het voorwerp dat ze zien zodra ze uit het ei zijn gekomen. In de meeste gevallen zal dat hun moeder of één van hun nestgenoten zijn. De jongen ontwikkelen dus de herkenning van hun ouders, en daarbij hun eigen soort, door te leren op een heel jonge leeftijd, en ze gebruiken deze kennis later in allerlei sociale contexten. Wanneer deze ingeprente kennis de omgang met ouders of nestgenoten beïnvloedt spreken we van inprenting van de volgreactie (in het Engels 'filial imprinting').Inprenting kan op een latere leeftijd ook de partnerkeuze beïnvloeden; in dat geval spreken we van seksuele inprenting. In sommige gevallen beïnvloedt inprenting, bij territoriale mannetjes, ook de herkenning van rivalen, in welk geval we van rivaal inprenting spreken. Hoewel inprenting van de volgreactie en seksuele inprenting vergelijkbare vormen van leren kunnen zijn, gebeurt het leren vaak niet op het zelfde moment, en zijn ze daarom waarschijnlijk twee aparte processen. Beide vormen van inprenting zijn wijdverbreid in vogels. Meer recent is seksuele inprenting ook gevonden bij zoogdieren, en ook in mensen. De wijde verspreiding van inprenten geeft aan dat het een standaard mechanisme van ontwikkeling van soortherkenning kan zijn in veel verschillende groepen van dieren, echter bij vissen is het tot nu toe nog niet onomstotelijk vastgesteld. Dat is ook geen wonder want de meeste soorten vertonen geen broedzorg waardoor jongen weinig kennis van soortgenoten op kunnen doen.

Het broedgedrag van cichliden uit het Victoriameer

De broedgewoonten van haplochromine cichliden maken het mogelijk dat hun jongen inprenten, omdat in alle soorten haplochromine cichliden vrouwtjes broedzorg vertonen. Mannetjes vertonen geen broedzorg, maar verdedigen hun territoria en baltsen naar langskomende vrouwtjes. Wanneer een vrouwtje op een baltsend mannetje af gaat, leidt het mannetje haar naar het midden van zijn territorium, waar het vervolgens tot paren kan komen. Tijdens het paren zwemmen het mannetje en vrouwtje al baltsend in rondjes achter elkaar aan. Als het vrouwtje besluit haar eitjes te laten bevruchten door het mannetje legt ze een eitje steeds even op de bodem waarna het mannetje het snel bevrucht. Het vrouwtje neemt het bevruchte eitje dan in haar mond, en daarna legt ze het volgende eitje. Vrouwtjes leggen vaak niet al hun eitjes bij een mannetje: tot vier mannetjes kunnen één broedsel bevruchten. Nadat het vrouwtje de eitjes in haar bek heeft genomen, verlaat ze het territorium van het mannetje en houdt de eitjes in haar bek tot ze zijn uitgekomen. Dit duurt zo'n vier weken. Als de vissenlarven hun dooierzak kwijt zijn, verlaten ze de mond van hun moeder en beginnen ze zelf hun voedsel te zoeken. De moeder blijft echter nog in de buurt en zal haar kroost ter bescherming terug in haar bek nemen bij dreigend gevaar. Deze uitgebreide broedzorg door het vrouwtje geeft de jongen in theorie de kans om op haar ingeprent te raken.

Rivaal herkenning en de co-existentie van soorten

Het stabiele voortbestaan van nauw verwante soorten in één gebied, zoals in het geval van de cichliden, vereist niet alleen dat ze reproductief geïsoleerd zijn. Er moet ook iets zijn wat ervoor zorgt ervoor dat de ene soort de andere er niet uit concurreert. Zelfs als de soorten reproductief geïsoleerd zijn, en net zo goed aangepast aan hun leefomgeving, dan nog voorspelt de theorie dat door toevallige fluctuaties in de populaties een van de twee soorten uitsterft. Meestal specialiseren soorten zich elk op een bepaalde voedsel bron. De minimale ecologische specialisatie van sommige soorten cichliden, zoals de soortenparen die ik als model soorten gebruik, is dan ook een merkwaardige situatie. Deze schijnbaar paradoxale situatie kan in theorie opgelost worden door te veronderstellen dat de relatief zeldzamere soort een voordeel heeft boven de relatief veel voorkomende soort, zodat de beide soorten elkaar in evenwicht houden. In het geval van de cichliden is geopperd dat territoriale gevechten voornamelijk tussen mannetjes van dezelfde soort voorkomen, en dat de hoeveelheid agressie die een zeldzaam mannetje ontvangt lager zou kunnen zijn. Dit voordeel zorgt er dan misschien voor dat het meer kan investeren in baltsgedrag, of ze zijn aantrekkelijker voor vrouwtjes, doordat ze in een betere conditie zijn. Ze zouden vaker kunnen paren, en de zeldzame soort zou minder zeldzaam worden. Echter, deze verhoogde kansen op paringen zouden dan wel met vrouwtjes van hun eigen soort moeten zijn, omdat anders de reproductieve isolatie niet in stand gehouden wordt. Een voorspelling van deze hypothese is dat mannetjes hoofdzakelijk het soorteigen fenotype als rivaal herkennen. Een van de mechanismen waardoor een soortgenoot als rivaal herkend wordt is 'rivaal inprenting', waarbij de jongen inprenten op het fenotype van de moeder.

Cichliden vertonen inderdaad voornamelijk agressie naar de eigen soort: als ze uitgedaagd worden om agressie te vertonen naar twee indringers, een van de eigen soort en een van een andere soort, zullen ze het merendeel van hun aanvallen naar de indringer van hun eigen soort richten. Hun territoria grenzen vaak aan dat van een mannetje van een andere soort, een patroon dat verwacht wordt als de 'zeldzame mannetjes hypothese' klopt. We weten echter niet of mannetjes ook daadwerkelijk kunnen profiteren van een verminderd aantal gevechten en of dit hun kans op het krijgen van nakomelingen verhoogt. Daarom is de 'zeldzame mannetjes hypothese' ook voorlopig nog niet meer dan een hypothese. Dit belet ons echter niet om te bestuderen hoe mannetjes hun rivaal herkenning ontwikkelen, omdat ons dit zal helpen te begrijpen of dit scenario in principe op zou kunnen treden in diverse stadia van soortvorming.

De hoofdstukken in dit proefschrift

In dit proefschrift stel ik de volgende vragen: Ontwikkelt soortherkenning bij cichliden uit het Victoriameer onder invloed van vroege ervaringen? En, als ze dat doen, kan dit het ontstaan van reproductieve isolatie onder sympatrische omstandigheden bevorderen? Dit onderzoek ik zowel in de context van partnerkeuze als rivaal herkenning. De vragen worden beantwoord in vier hoofdstukken, waarin ik de effecten van inprenten in de context van sympatrische soortsvorming bekijk met wiskundige modellen, en met experimenten met individuen van de soortenparen die ik hierboven heb beschreven.

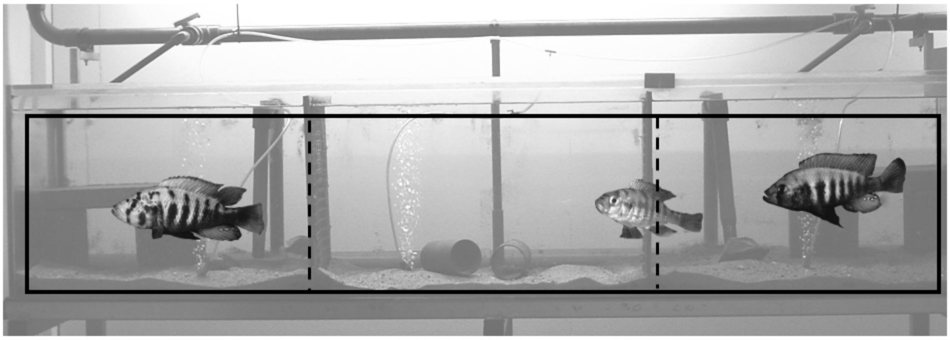
Het eerste hoofdstuk is een algemene inleiding tot het onderwerp, waarop deze Nederlandse samenvatting gebaseerd is.

In hoofdstuk twee vergelijk ik hoe goed de verschillende manieren waarop dieren een voorkeur voor partners van de eigen soort kunnen ontwikkelen zijn in het in stand houden van reproductieve isolatie. De verschillende mechanismen van ontwikkeling van soortherkenning zijn: een door genen bepaalde voorkeur; een voorkeur voor een partner met hetzelfde fenotype als het individu zelf heeft; seksueel inprenten op de moeder; seksueel inprenten op de vader; en tenslotte een vorm van inprenten op een willekeurig individu uit de populatie. Ik laat zien dat de manier waarop vrouwtjes hun partnervoorkeur ontwikkelen van grote invloed erop kan zijn of nieuwe soorten kunnen vormen zonder een geografische barrière. Soortvorming gaat weliswaar relatief makkelijk als vrouwtjes automatisch een voorkeur hebben voor hun eigen fenotype, maar dit mechanisme komt wellicht bij erg weinig soorten voor. Deze uitkomst is goed te vergelijken met het model waarin vrouwtjes op hun moeder inprenten. Verrassend genoeg is soortvorming een stuk moeilijker als vrouwtjes niet op hun moeder maar op hun vader inprenten. Dit komt waarschijnlijk doordat het aantal nakomelingen dat een mannetje kan krijgen afhankelijk is van de vrouwtjes die voor hem kiezen, waardoor het fenotype dat net iets meer nakomelingen krijgt in de volgende generatie nog weer populairder is. Dit werkt soortsvorming danig tegen, aangezien zo het ene fenotype het andere er makkelijk uit concurreert. Wanneer vrouwtjes niet op een van de ouders inprenten, maar op een individu dat ze tegenkomen als ze willen paren (dus eigenlijk een willekeurig individu uit de populatie), dan wordt soortvorming onmogelijk. Tenslotte, wanneer vrouwtjes afhankelijk zijn van hun 'voorkeurs genen', dan kunnen de twee vormen van het mannelijke broedkleed beiden makkelijk in de populatie blijven, maar de koppeling tussen het veranderde kenmerk en de voorkeur ervoor ontstaat dan niet makkelijk, en de interpretatie van deze situatie als soortvorming zou dubieus zijn. De conclusie van dit model is dat het voor soortvorming wel degelijk uitmaakt hoe de partnervoorkeur zich ontwikkelt, en dat inprenten op de moeder de tot standkoming van reproductieve isolatie relatief makkelijk maakt.

Het derde en vierde hoofdstuk zijn onderdeel van hetzelfde experiment, in hoofdstuk drie beschrijf ik experimenten met vrouwtjes, in hoofdstuk vier met mannetjes. Ik heb een adoptie experiment opgezet om te testen voor de effecten van seksueel inprenten in de soorten *Pundamilia pundamilia* en *Pundamilia nyererei*. Hiervoor heb ik de eitjes verwisseld tussen de broedende vrouwtjes, zowel tussen vrouwtjes van dezelfde soort, als tussen vrouwtjes van twee verschillende soorten. De broedende vrouwtjes accepteerden zo'n 50% van de tijd het vreemde broedsel, en dan was de adoptie dus gelukt. De adoptiemoeders konden daarna hun kroost opvoeden zoals dat normaal ook gebeurt.

In hoofdstuk drie test ik de partnervoorkeur van de inmiddels volwassen geworden

Figuur 1 Een vrouwtje ontvangt haar adoptie eitjes.
Op deze foto heb ik in mijn linkerhand een vrouwtje (haar bek is net te zien). In mijn rechterhand heb ik een plastic pipet, waarin ik de eitjes van een ander vrouwtje heb opgezogen. Ik knijp de pipet leeg, waardoor de eitjes in de bek van het vrouwtje terecht komen.



Figuur 2. Het testen van de partnervoorkeur van een vrouwtje. Het vrouwtje heeft de keuze tussen twee mannetjes, een van elke sort. De mannetjes kunnen niet door de mazen van het gaas (aangegeven met de stippellijn), maar het vrouwtje is kleiner en kan vrij bewegen door het aquarium.

geadopteerde dochters. De vrouwtjes konden vrij bewegen in de territoria van twee mannetjes, een van elke soort, terwijl ik hun baltsgedrag observeerde. De vrouwtjes die opgegroeid waren bij een moeder van de andere soort hadden ook een voorkeur voor de mannetjes van die andere soort, en hun voorkeur was significant anders dan die van de vrouwtjes die bij een adoptie moeder van hun eigen soort waren opgegroeid. Dit laat zien dat de partnervoorkeur van de vrouwtjes zich ontwikkelt door op een vroege leeftijd over hun moeders fenotype te leren. Seksueel inprenten in cichliden kan dus bijdragen aan de reproductieve isolatie tussen verschillende fenotypes zonder dat de genetische achtergrond voor partnerkeuze hoeft te veranderen. Gecombineerd met de resultaten uit hoofdstuk twee, doe ik de suggestie dat het voorkomen van dit inprentmechanisme erg belangrijk is geweest in de evolutie van de opmerkelijke soortenrijkdom van de Oost Afrikaanse cichliden.

Het vierde hoofdstuk beschrijft de effecten van het adoptie experiment op het gedrag van de mannetjes. Ik test of vroege ervaring zowel de partnerkeuze voorkeur van mannetjes beïnvloedt, als de mannelijke rivaal herkenning. Deze beide testen werden gedaan in twee-keuze testen, waarbij de mannetjes steeds konden kiezen uit individuen van beide testen werden gedaan in twee-keuze testen, waarbij de mannetjes steeds konden kiezen uit individuen van beide soorten. Onafhankelijk van welke soort adoptiemoeder de mannetjes opgegroeid waren, richtten ze allemaal hun agressie naar indringers van hun eigen soort. Ook de partnervoorkeur bleek onafhankelijk te zijn van de soort adoptie moeder. Ik concludeer hieruit dat mannetjes van deze soorten hun soortherkenning ontwikkelen zonder de invloed van vroege leer ervaringen. Dit staat in schril contrast met de conclusie uit hoofdstuk drie, waarin ik heb laten zien dat de vrouwtjes juist wel seksuele inprenting vertonen. Echter, ik heb wel een indicatie gevonden dat territoriale ervaring de voorkeur voor het aanvallen van de eigensoort versterkt.

Het vijfde hoofdstuk gaat over een adoptie experiment met het tweede soorten paar, *Mbipia mbipi* en *Mbipia lutea*. Dit experiment test niet alleen voor een effect van het fenotype van de moeder op de ontwikkeling van soortsherkenning, zoals in het *Pundamilia* soortenpaar, maar test ook of er een effect is van de ervaring met de broedsel genoten. In dit experiment heb ik daartoe ook gemengde broedsels gemaakt, door ofwel maar een paar eitjes te verwisselen, of de meeste, maar niet alle eitjes. Ik heb getest op de effecten van ervaring met de fenotypes van de adoptiemoeder en de nestgenoten, op zowel de partnervoorkeur van de vrouwtjes, als de ‘agressievoorkeur’ van de mannetjes. Net als in hoofdstuk drie, laat ik hier zien dat de voorkeur van de vrouwtjes sterk beïnvloed wordt door leren over het fenotype van hun adoptieve moeder. Daarbij laat dit experiment ook zien dat de partnervoorkeur van de vrouwtjes niet beïnvloed wordt door ervaringen met nestgenoten, terwijl ze daar in dit experiment wel in ruime mate mee in aanraking zijn gekomen. De rivaalherkenning van de mannetjes daarentegen, wordt wel beïnvloed door de ervaring met nestgenoten, maar niet door het leren over hun moeders fenotype.

Conclusie

Ik concludeer na deze experimenten dat de partnervoorkeur van vrouwtjes cichliden beïnvloed wordt door de ervaring op vroege leeftijd met hun moeders uiterlijk. Hiervoor heb ik bewijs geleverd met experimenten met twee verschillende soortenparen. De overeenkomst van dit effect tussen de twee experimenten geeft aan dat dit gedrag bij meer soorten haplochromine cichliden voor zou kunnen komen. Hiermee verbreden we dus de reikwijdte van onze bevindingen en het begrip van de soortenrijkdom in het Victoriameer. Bovendien geeft het aan dat gedrag van de twee *Pundamilia* soorten, waar ook diverse andere experimenten mee gedaan worden in het kader van soortsvorming bij cichliden, inderdaad representatief zou kunnen zijn voor de soortengroep van cichliden uit het Victoriameer.

In tegenstelling tot de vrouwtjes, blijkt de soortherkenning van de mannetjes, niet door inprenten op de moeder beïnvloed te worden, maar de rivaalherkenning wordt wel beïnvloed door de ervaring met hun nestgenoten. In het wild zouden de mannetjes niet alleen van hun nestgenoten kunnen leren, maar ook van andere mannetjes die ze tegenkomen. Deze andere mannetjes kunnen van een andere soort zijn, en dit zou het onwaarschijnlijk maken dat individuen vooral hun eigen soort als rivaal gaan herkennen. De voorspelling van de ‘zeldzame mannetjes hypothese’ lijkt dus niet uit te komen, en het is daarmee onwaarschijnlijk geworden dat de relatief zeldzame soort een voordeel heeft door verminderde agressie. De co-existentie van de minimaal gespecialiseerde soorten blijft dus nieuwe vragen oproepen.

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Curiculum Vitae

Ik ben op 13 september 1976 in Leiden geboren. Tussen 1988 en 1994 doorliep ik het VWO, dat ik afsloot met het diploma van het Zandvliet college, te Den Haag. In datzelfde jaar begon ik aan de studie bosbouw aan de Wageningen universiteit, maar na een blauwe maandag ben ik van studierichting veranderd en aan filosofie begonnen, in Leiden. In het volgende jaar, 1995, ben ik biologie gaan studeren, aan dezelfde universiteit. Al snel werd mij duidelijk dat mijn interesse lag bij de studie van het gedrag, en mijn beide doctoraal stages heb ik dan ook bij de sectie gedragsbiologie gedaan, onder supervisie van Hans Slabbekoorn, Selvino de Kort en Carel ten Cate. De laatste van deze twee stages gaf mij de kans veldwerk te doen in Kameroen, en in januari 1999 ben ik, samen met Paula den Hartog, naar Afrika vertrokken, waarmee een lang gekoesterde droom in vervulling ging. Na terugkeer van dit avontuur heb ik mijn veldvaardigheden uitgebreid als veldassistent bij Rita Covas in Zuid Afrika, bij haar onderzoek aan de life history van de ‘sociable weaver birds’. Na mijn afstuderen in 2000, ben ik twee seizoenen achter elkaar wederom veldassistent geweest, voor Rob Lachlan bij zijn project aan de vinken op de Canarische eilanden. In augustus 2001 begon ik als junior onderzoeker bij de sectie gedragsbiologie. In deze periode heb ik het fenomeen peak-shift onderzocht aan twee kenmerken van zebravinken: de geleerde voorkeur voor snavelkleuren en de geleerde herkenning van zebravinken liedjes. Eind 2002 begon ik aan mijn promotieonderzoek aan de effecten van vroege ervaring op het seksuele gedrag van de cichliden uit het Victoriameer, waarvan dit proefschrift het resultaat is. Na mijn promotie vertrek ik naar de VS voor een postdoc baan aan de universiteiten van Texas A&M en North Carolina te Chapel Hill bij de labs van respectievelijk Gil Rosenthal en Maria Servedio.

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