Evolutionary mechanisms of population divergence in Eurasian perch (*Perca fluviatilis* L.)

Doctoral Thesis

Dissertation

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Chapter 1

Introduction

> *. . . whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been and are being, evolved.*
>
> (Charles Darwin, 1859)

Charles Darwin set a milestone for the study of evolution as a science by publishing his book *The origin of species by means of natural selection* in 1859 (Darwin, 1859). He was convinced that variation existed between individuals belonging to one species. This variation could, under certain circumstances like e.g. environmental changes, lead to an advantage of some individuals over others giving them better chances of survival and of "procreating their kind". Other variations however, could be deleterious for individuals. The preservation of favorable, and the destruction of deleterious variations he called natural selection. In consequence, Darwin believed that natural selection, as a kind of natural law, was the driving force of evolution and responsible for the enormous species diversity on earth. In the following decades, Darwin's theory was controversially discussed, neglected and finally rediscovered, due to an increasing amount of theoretical and empirical studies, proving the existence and fundamental relevance of natural selection for evolutionary processes (for a review see Ayala & Fitch, 1997). A relatively new scientific field emerged, evolutionary biology. The central question of evolutionary biology is the understanding, how diversity on earth came about. Following the discovery of the DNA structure by Watson and Crick in 1953, effective tools for all kinds of genetic studies were developed rapidly. This gave rise to many differing disciplines within evolutionary biology. Molecular evolutionists and Systematists both are interested in phylogenetic trees but while the former think about history that is recorded in DNA sequences, Systematists concentrate on variation among species. Palaeontologists work in large time scales and concentrate on large-scale trends (macro evolution). Population and

quantitative geneticists concentrate on micro evolutionary processes that occur over relatively short time periods within populations and investigate factors, maintaining genetic variation. Evolutionary ecologists finally avoid genetic details and investigate phenotypic variation and its influence on the reproductive success of individuals (Stearns & Hoekstra, 2001).

This study aims in understanding evolutionary processes that drive population subdivision, as a potential basis for speciation in the future. It concentrates on micro evolutionary processes and is thus mostly dedicated to population genetics and evolutionary ecology (Stearns, 2001). I investigated causes for population subdivision, focusing on populations living in sympatry.

In contrast to many terrestrial species, freshwater fishes are excellent model organisms to study variation between populations because they are often subdivided into defined local populations, isolated by insuperable terrestrial areas. Genetic drift and/or local adaptations to ecological factors such as specific temperature or light conditions or resource abundance might lead to morphological and/or behavioral changes in one population that prevents it from interbreeding with another. The resulting reproductive isolation can drive further genetic divergence. Similar processes can occur between or within river systems where different flow regimes or water temperatures can result in different ecological niches even within one river (Carvalho, 1993). It could be shown that population subdivision can occur sympatrically, without geographical isolation of populations (Via, 2001). The most well known example in freshwater fishes is the evolution of the enormous cichlid species richness in East African lakes, which have formed not only very rapidly but also without spatial isolation (Meyer et al., 1990; Johnson et al., 1996). Recent studies have shown that divergence under sympatric conditions had also played a major role in stickleback (reviewed in Schluter, 1996) and in whitefish speciation (Lu & Bernatchez, 1999).

Analysis on parasite infection in Eurasian perch (*Perca fluviatilis* L.) of Lake Constance showed differences in the intensities of infection rates between capture sites (Balling & Pfeiffer, 1997; Dieterich, 1998). This was the first indication of a possible population subdivision within lake basins. According to these results, Gerlach et al. (2001) investigated the genetic structure of perch using microsatellites and found that they are subdivided into two genetically distinct subpopulations between lake basins.

The present study investigates evolutionary mechanisms that could have caused reproductive isolation of the two subpopulations and may drive speciation in perch. In the following paragraphs of the introduction, I first describe and discuss species

concepts that may help us to define, what species are. Thereafter I will give a short discourse on speciation, one of the central processes in species evolution. Afterwards I argue how natural selection and its derivates sexual- and kin selection can drive speciation. Finally I will formulate the aims of this study in detail.

1.1 Identification of species, the biological species concept (BSC) and the phylogenetic species concept (PSC)

We discriminate living things by various traits like e.g. color or coat patterns, and group resembling individuals to species. Anyone can discriminate between a leopard and a tiger, although the individuals of these related species have many traits in common. Some traits e.g. coat pattern varies more between species than within them, indicating that the species unit seems to be a natural one. However, this method to classify species is often misleading, because discriminating traits between two species can overlap or several populations of species can differ in specific traits, what we call phenotypic plasticity. Even hybridization between species can occur. So what can we do to identify species? What have individuals belonging to the same species in common?

There are two concepts that may help us. The earliest one is the **biological species concept (BSC)** advanced by Dobzhansky (1937) and propagated by Mayr. It says that "species are groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups" (Mayr, 1942; 1963). The BSC is very powerful in sustaining different species because it is based on successful sexual reproduction. On one hand, a species maintains its uniformity by genetic recombination of freely interbreeding individuals within the common gene pool. On the other hand, gene flow between different species is prevented because their individuals can or do not mate successfully. But the BSC is not applicable to all cases: it does not hold true for asexual species e. g. the imperfect fungi, where genetic recombination does not occur, or for interspecies hybridization, where individuals of different species mate and produce fertile offspring. Hybridization is not very common in animals but frequently occurs in plants and fungi. Here the question is, how much gene flow between species is acceptable to still talk about different species according to the BSC.

In order to overcome the challenges that arise when using the BSC to define species Cracraft developed the **phylogenetic species concept (PSC)**. The PSC defines a species as a monophyletic group composed of "the smallest diagnosable cluster of individual organisms within, which there is a parental pattern of ancestry and descent" (Cracraft, 1983). This method takes care of the historic dimension,

since every individual inherits certain characters from its ancestors by descent. According to the PSC, species are groups of individuals that share certain derived characters, which distinguish them from other comparable groups. Since it is not clear however, how many derived characters define a new species in this model, the PSC is also very problematic in practice. With high-resolution molecular methods, well established species would split up into several groups of individuals that share specific characters, and this of course is not very useful. Every newly developed character would then produce a new species and species number would increase dramatically.

Despite the extensive scientific literature on the definition of species and the development of abstract species concepts as shown above, evolutionary biologists still disagree on this issue. Therefore taxonomists use all sorts of differences (morphological, behavioral and genetic differences), to identify species. Discussing species concepts shows that our definition does not define a species uniquely. There are often serious problems to decide how strong populations of a taxonomic unit must differ to be classified as separate species. For sexually reproducing animals the BSC is more applicable and thus is widely used.

1.2 Speciation

Considering the amount of different species on earth the question arises, how this diversity might come about, how existing species have evolved. The splitting of populations into evolutionary independent units, which is the basis of species formation, is called **speciation**. Speciation occurs as a by-product of selection and drift operating on individuals (Stearns & Hoekstra, 2001). The same processes that cause genetic diversity within a population are responsible for their divergence. Mutations are the ultimate cause of genetic diversity. They can change the genetic code, which will increase variation between individuals. If mutations occur, natural selection (see Section 1.3) or genetic drift can cause the spreading of new traits within the population. Beneficial new traits may help some individuals to adapt better to certain ecological niches, and new ecotypes evolve. We call this process ecological speciation (for a review see Schluter, 2001). If the gene flow between populations is interrupted due to geographical isolation, to local habitat selection, to differing resource use or to mate selection, selection can act on subpopulations that will slowly split up. If enough time passes and the populations depart in a way that after secondary contact mating does not occur because of sexual incompatibilities, differences in mating time, different mating behavior or unfit or sterile hybrid offspring, the basis for the formation of distinct species is set. We know of two main scenarios that could cause

species formation: allopatric and sympatric speciation.

1.2.1 Allopatric speciation

For a long time, the **allopatric speciation model** by Mayr (1963) was the most widely accepted one. In this model speciation is caused by geographical isolation of populations. Geographic barriers can be due to geological events, to the extinction of intervening populations, or to the migration of individuals e. g. to islands that are separated by water. If time passes by, genetic differences accumulate due to genetic drift and/or different selective forces acting on the separated populations. The result can be reproductive isolation that prevents cross matings, even if populations meet secondarily. According to this model, the first step in speciation is for one population to split up into two or more completely isolated subpopulations.

1.2.2 Sympatric speciation

In the **sympatric model**, speciation occurs without prior population splitting. Geographic and ecological conditions can lead to a partial isolation of subpopulations. The main question is, whether subpopulations can become reproductively isolated despite the presence of some gene flow.

First empirical evidence for sympatric speciation was given by the experiments of Bush and co-workers in *Rhagoletis pomonella* flies (Bush, 1994). They could show that these parasitic insects made a host shift from hawthorn to apple and later to other fruits, which resulted in the evolution of genetically different races of flies.

This form of sympatric speciation involves reproductive isolation as a result of adaptation to discrete resources and/or habitats. Because of the lack of an intermediate habitat (ecological discontinuity), where hybrids might survive, ecological selection acts against hybrid offspring, which is less well adapted to both parental habitats (Via, 2001).

A more recently described type of sympatric speciation is caused by direct disruptive selection (see next paragraph) on traits associated with continuously distributed resources and/or by sexual selection against a continuum of phenotypes. In this case, disruptive selection acts against intermediate phenotypes, which fail to compete successfully for continuously distributed resources (Turelli, 2001). Schluter and colleagues could show that intermediate phenotypes of threespined sticklebacks had lower growth rates in the presence of a specialized competitor (Schluter, 1994). In addition, hybrids of two stickleback species, a benthic and a limnetic one, had lower growth rates in both parental habitats (Schluter, 1995; Hatfield & Schluter, 1999). Hybrids or intermediate phenotypes might also suffer fitness losses in the

form of lower reproduction success, because sexual selection favors extreme phenotypes. Sexual selection as driving force for sympatric speciation is well investigated in many species, and can very rapidly lead to reproductive isolation. The enormous species richness of the haplochromine cichlid species complex in Lake Viktoria is a spectacular example. The lake contains about 500 to 1000 haplochromine cichlid species that very likely have formed within less than 13,000 years in one continuous water body (Meyer et al., 1990; Johnson et al., 1996). In haplochromines, males of sympatric species pairs show different color morphs. Seehausen and colleagues could show that female choice for male color causes reproductive isolation between sympatric species. If they masked color differences in laboratory experiments (under monochromatic light) mating preferences disappeared (Seehausen & Van Alphen, 1997, 1998). Further empirical support for sympatric speciation has come from studies on monophyletic cichlid species pairs in crater lakes of Cameroon. Schliewen and colleagues could show that differential ecological adaptations in combination with assortative mating could easily lead to speciation in sympatry (Schliewen et al., 1994, 2001).

These empirical studies have motivated numerous authors to develop theoretical models supporting the plausibility of sympatric speciation. Three classes of models have been provided (after Turelli, 2001): ecological models, based on competition for resources (Dieckmann & Doebeli, 1999; Kondrashov & Kondrashov, 1999; Drossel & McKane, 2000), sexual selection models, based on competition for mates (Higashi et al., 1999; Turner & Burrows, 1995), and models of habitat-race formation that are based on habitat specific deleterious or beneficial alleles (Kawecki, 1996; Kawecki, 1997). Here I will shortly introduce two of the ecological models, the ones of Kondrashov and Kondrashov and Dieckmann and Doebeli following the excellent description by (Tregenza & Butlin, 1999). Imagine a lake that contains two potential food resources, large and small prey. In a population of predatory fish, big and small fish will do best in using the respectively sized resource while fish of intermediate size will be at disadvantage. This disadvantage to intermediates is termed **disruptive selection**. It causes a strong selective pressure for the population to split into two subpopulations containing distinct ecological types. However, subpopulations containing big or small fish will not form, as long as matings between distinct ecotypes mixes their genes. Taking care of this problem, both theoretical models require the evolution of genetic associations between ecologically important traits and neutral markers, which are used for mate choice like e.g. a preference for a specific color morph. In the model of Kondrashov and Kondrashov, starting from an intermediate uniform population (intermediate size and uniform color but genetic variation for

both traits), disruptive selection favors the most extreme phenotypes. Matings in this model occur either between mates that are most similar for a single neutral marker trait (similar in color) or it depends on a match between female preference for a distinct male trait (one male color morph is preferred regardless of the female's own color). The population will split when selection increases associations between ecological traits and marker traits. If this happens, it will lead to assortative mating between distinctly colored ecotypes e.g. between yellow ecotypes with big gapes and blue ecotypes with small gapes. Due to reproductive isolation, genetic differences can now accumulate. In the model of Dieckmann and Doebeli, disruptive selection for distinct ecotypes arises explicitly due to competition for a single resource. Here the starting point is a unimodal distribution of the resource. A population of individuals with one particular phenotype will do best in using this resource as long as its carrying capacity is high enough. As soon as the population approaches the resource maximum, competition between similar individuals creates selection, which favors phenotypes that are able to use slightly less abundant resources but can compensate for this sub-optimal state by a decrease in competition. Assortative mating driven by associations between the ecological and a marker trait can now lead to sympatric speciation similar to the model of Kondrashov and Kondrashov.

1.2.3 Hybridization and speciation

The role of hybridization in evolution is discussed controversially. Zoologists consider hybridization problematic for speciation and taxonomy, since it causes a mixture of discrete gene pools. Botanists on the other hand argue that hybrids can contribute to genetic variation and in some cases can give rise to new recombinant species (Barton, 2001). However, recent studies have shown that hybridization has also contributed to the evolution of animals (Dowling & Secor, 1997). New additive genetic variation can be introduced into the gene pools of distinct species of Darwin's finches by hybridization, and this could facilitate evolutionary change (Grant & Grant ,1994).

In the following I will focus on mechanisms that prevent hybridization, drive reproductive isolation of subpopulations, and thus reinforce speciation. Cross matings between individuals of two closely related species or subpopulations can be very costly for the mating partners, mostly because of fitness losses when producing unfit hybrid offspring. The result can be postzygotic isolation, of, which two forms have been distinguished (Rice & Hostert, 1993; Coyne & Orr, 1998; Schluter, 1998). Hybrids inherit a mixture of traits from their parental populations and can show an intermediate phenotype. If an interaction exists between the phenotype and the environment, hybrids with an intermediate phenotype may not be fit in either of

the parental environments and are selected against (termed "environment-dependant postzygotic isolation" by Rice and Hostert, 1993). Intrinsic genetic isolation (termed "unconditional isolation" by Rice and Hostert, 1993) occurs independently of the environment. In this case, hybrids have a reduced viability and/or fertility due to genetic incompatibilities between the parental genomes.

Due to the high costs of hybridization, Dobzhansky considered already in 1940 the evolution of mechanisms that convert postzygotic to prezygotic isolation. This process is known as secondary reinforcement (Dobzhansky, 1940). Prezygotic isolation occurs, if mechanisms evolve that prohibit hybridization and lead to reproductive isolation. A very powerful mechanism that can avoid hybridization and can drive speciation is assortative mating (Kondrashov & Shpak, 1998; Kirkpatrick, 2000). Assortative mating occurs, if individuals prefer mating partners with a similar phenotype to their own. It is frequently based on body size, morphology and habitat choice. Studies on sexual selection against hybrids of two forms of sympatric sticklebacks indicate that assortative mating had contributed to speciation in this fish species (Hatfield & Schluter, 1996; Vamosi & Schluter, 1999). In sticklebacks, assortative mating seems to be based on body size (Nagel & Schluter, 1998). Evidence for secondary reinforcement could also be found in the extended studies on sympatric *Drosophila* species. Coyne and Orr (1989; 1997) showed that both, prezygotic and postzygotic isolation increase with divergence time between allopatric and sympatric species pairs, whereby prezygotic isolation evolves more rapidly than postzygotic isolation. However, prezygotic isolation was found to be much stronger between sympatric than between allopatric taxa of *Drosophila*. All of the investigated sympatric species pairs of *Drosophila* and also of the sticklebacks discussed above were initially allopatric species that had come into secondary contact (Coyne & Orr, 1997; Rundle & Schluter, 1998), which is the pre-requisite of secondary reinforcement as defined by Dobzhansky. To my knowledge, nothing is known about evolutionary rates of postzygotic and prezygotic isolation in species pairs that have developed in "true" sympatry without an initial allopatric phase.

1.3 Natural selection and its derivates: sexual, and kin selection

Darwin, who gave the first definition of natural selection, was at his time not able to explain, what forces cause new traits to arise, and how these are inherited from one individual to its offspring. Today, we know that it is mutations that can produce new genes. If these lead to beneficial traits for the individual in the form of more and/or fitter offspring, the genes will spread within the shared gene pool of a population. This selective process that accounts for the adaptation of organisms to their envi-

ronments, we call **natural selection** (which is not so far from Darwin's definition in 1859). Thus natural selection will always favor individuals with characteristics increasing their survival and the number and fitness of their offspring. However, there will never be "the best adapted genotype", since environments always change and new mutations giving rise to better adapted individuals can develop at any time.

In sexually reproducing organisms, differences almost always exist in the parental investment between males and females (Trivers, 1972). The production of eggs is very costly because eggs contain a high amount of nutrients for embryonic development, whereas sperm contains almost solely the male genes. Additionally females often invest more time for parental care than males. This leads to differences in the reproductive potential of the different sexes with females mostly having a lower reproductive potential then males (Clutton-Brock & Vincent, 1991). The result can be that at the same time the number of receptive females is much lower than that of receptive males (operational sex ratio by Emlen & Oring, 1997). Under these circumstances males have to compete for females whereas females can choose between males. These two processes are the basis of **sexual selection,** which is a derivate of natural selection. In sexual selection, traits are selected that are directly associated with reproduction and partner choice. There are two hypothesis that try to explain, how female choice can influence the evolution of elaborate male secondary sexual traits like e.g. the bright male coloration and ornamentation in many fish species. The first is the "run away selection" or "sexy son" hypothesis (Fisher, 1930). Consider a population, were variability exists for specific male traits, e.g. for male ornamentation. According to Fisher's hypothesis, a female preference for a specific male ornamentation arises. Females will prefer to mate with males that carry the preferred trait. If both traits (the preference and the preferred ornamentation) are heritable, male and female offspring of a couple may carry genes for both traits. The result can be a covariance between both genes, were the preference is pronounced only in females, and the preferred ornamentation in males. The process is self- reinforcing. Every time, a mutation arises that increases female preference for e.g. a brighter male ornamentation, brighter ornamented males will spread throughout the population. Additionally, due to competition between males, the intensity of the ornamentation will increase until natural selection against traits that are too elaborate or risky will stop the process.

In contrast, the "handicap" or "good genes" hypothesis sees elaborate male traits as true indicators for "good genes" (Zahavi, 1975). Elaborate male traits can display a handicap for daily survival. The bright ornamentation of males could signal a predator that there is available prey. Therefore, males that live in spite of the

"handicap" should have "good genes" for other qualities that increase their survival. Females should choose these males, because they will produce offspring with a high potential to survive.

Sexual selection acts directly on the fitness of individuals. Therefore, it can lead to speciation very quickly. The classic fish example for sexual selection is the extreme diversity and rapid species formation within the East African cichlids (Seehausen & van Alphen, 1999). In these fish species repeatedly and independently in different lakes, females developed preferences for specific male color morphs leading to assortative mating, although reproduction with the "wrong" color morph was still possible (Seehausen & Van Alphen, 1997; Seehausen & Van Alphen, 1998).

In social organisms that interact with relatives, another kind of selection can be found, which we call **kin selection** (Hamilton, 1964). Kin selection occurs, if an individual increases its own fitness by helping close relatives to increase their reproductive success. Why should an individual do so? The background of kin selection is that some of the genes in the relative are identical to the individual's own genes. In helping the relative to reproduce, the individual's own genes are transmitted to future generations, which is the classical aim of reproduction. The closer the relatedness, the more likely that this behavior will be achieved. However, kin selection only occurs when the benefits exceed the costs for the helping individual. This insight led Hamilton to formulate the theory of kin selection, known as Hamilton's rule: $(br > c)$ where kin selection is favorable the fitness benefit for the recipient (b) times the relatedness of the recipient to the helper (r) is bigger then the fitness cost of the behavior to the helper (c).

Today we have many indications that kin selection gave rise to altruistic behavior, which can be seen in extreme form within the eusocial insects where only one individual (the queen in some bees and all ants and termites) reproduces and thousands or millions of sterile social helpers (mostly full or half sisters to the queen) help in raising the offspring on the cost of their own reproductive success (Seger, 1991). In fish, to my knowledge, kin selection has never been shown directly, but there is some strong evidence that it could also occur. Sticklebacks e. g. are more likely to conduct co-operative behavior with relatives during risky situations like predator inspection (Milinski, 1987). However, kin recognition and kin preference, which could be good indicators for putative kin selection (although kin selection can also be found without kin recognition) have been studied for many fish species (for a review see Krause et al., 2000). Pitcher and Parrish (1993) have shown that shoaling in fish provides direct fitness benefits for individual shoal members. Shoaling with distinct individuals, matched in size or color, or shoaling with familiar or related

individuals could additionally be advantageous (Krause et al., 2000).

A recent theoretical model of socially mediated speciation by Hochberg et al. (2003) shows, that altruistic acts could produce tribes that are reproductively isolated. According to this model, altruistic behaviors could lead to assortative mating between like phenotypes. Thus the results of Hochberg et al. (2003) show how social selection in the absence of divergent natural selection can establish the conditions for reproductive isolation, a well known basis for speciation.

1.4 Aims of the study

In this study I aim at investigating evolutionary mechanisms that can drive and maintain the splitting of populations living in sympatry.

In the first chapter I investigated if the origin of the two subpopulations of perch in Lake Constance is based on divergence in allopatry with secondary contact afterwards, or if the two populations have diverged sympatrically within the same water body. Therefore, mtDNA analyzes of perch belonging to the two subpopulations and of perch from adjacent waters including the two possible glacial refuges were performed.

Subpopulation structure can only be maintained by assortative mating within the distinct populations. Sexual selection, commonly known to result in assortative mating, is unlikely to play a role in perch subpopulation structure. Therefore, the second chapter deals with sensory capabilities of perch and investigates whether kin and population recognition based on olfactory cues exist. Furthermore, kin and population recognition could lead to assortative mating and drive population splitting. Kin recognition and kin preference could explain the close relatedness within shoals of juvenile and adult perch in the littoral zone of Lake Constance, found by Gerlach et al. (2001). An additional microsatellite analysis on the genetic structure of free ranging shoals of perch larvae shall show, if perch form kin aggregations already during this highly mobile pelagic phase.

Hybridization between distinct populations can on the contrary lead to further population splitting. Therefore, the subject of the third chapter is a study on hybrid fitness parameters to find out, whether a reduction in hybrid fitness could reinforce population divergence in perch.

The results of a preliminary study on morphological differences between the two subpopulations are given in chapter four. Such differences could indicate that different ecotypes may have formed in adaptation to distinct habitat conditions.

In the final discussion, general conclusions will be given, emphasizing that this study gives the first empirical evidence for a novel evolutionary mechanism driving

population divergence, which has so far been discussed only theoretically.

The sections of this thesis are or will be presented as independent publications, therefore some parts may recur. The following publications have been published slightly altered or will be submitted for publication.

Behrmann-Godel J, Gerlach G, Eckmann R 2004: Postglacial colonization shows evidence for sympatric population splitting of Eurasian perch (*Perca fluviatilis* L.) in Lake Constance. *Molecular Ecology*, **13,** 491-497.

Behrmann-Godel J, Eckmann R, Atema J, Gerlach G: Group and kin preference can drive speciation *submitted.*

Besides helpful discussions, my co-authors have contributed the following to this thesis:
Most of the different genetic analysis I performed in Gabriele Gerlach's laboratory and I highly profited by her experience and help in all kinds of genetic and behavioral investigations.
Jelle Atema was indispensable for developing the flume and the experimental set up that I used for the behavioral experiments in chapter one.

Chapter 2

Postglacial colonization shows evidence for sympatric population splitting of Eurasian perch (*Perca fluviatilis* L.) in Lake Constance

A previous microsatellite analysis showed that two subpopulations of perch (*Perca fluviatilis* L.) exist in Lake Constance (Gerlach et al., 2001). This raises questions of whether (i) Lake Constance had been colonized by two populations that had diverged in allopatry or (ii) the two subpopulations have diverged in sympatry. Sequence analysis of a 365 bp mtDNA fragment (5' end of the D-loop) of perch from Lake Constance and adjacent waters revealed ten haplotypes. We suggest colonization via the Danube river, based on the frequency and dispersion of haplotypes, and knowledge of the lake's paleohydrological development. Pairwise F_{ST}-values using mtDNA sequences showed no significant population subdivision. Our study provides strong evidence that subpopulations of perch in Lake Constance have diverged in sympatry.

2.1 Introduction

Pleistocene glaciations have not only widely formed today's landscapes, but have also had major influences on the patterns of animal dispersal and colonization (Avise, 1992; Hewitt, 1996; Bernatchez & Wilson, 1998; Taberlet et al., 1998; Hewitt, 2001). In Europe, wide areas of Scandinavia and the Alps were repeatedly covered by ice shields during the Pleistocene glaciations. Glacial advances and retreats have formed lakes and reorganized river channels and drainages. During the Pleistocene glaciations the Rhine glacier coming from the inner Alps excavated the deep prealpine Lake Constance. The earliest colonization of Lake Constance by fish could have taken place during the retreat of the most recent glacier 15,000 – 10,000 years ago.

The rivers Rhine and Danube, which are the two nearest fluvial systems, are the most probable refugia for freshwater organisms for Lake Constance. These two rivers are already known as refugia for genetically different forms of a variety of freshwater fish species including Eurasian perch (Riffel & Schreiber, 1998; Nesbo et al., 1999; Gross et al., 2001; Weiss et al., 2002).

Our study focused on Eurasian perch (*Perca fluviatilis* L.) a widely distributed freshwater fish species, which is the second most common one in Lake Constance. A previous microsatellite analysis had shown that perch in Lake Constance do not form a panmictic population but are subdivided into two genetically different subpopulations ($G_{ST} = 0.07$). One population is only found in the Upper Lake (population 1), which is deep, oligotrophic, and warm-monomictic. The other population is mainly found in the Lower Lake (population 2), which is much shallower, mesotrophic and dimictic. Both lake basins are connected by a stretch of lotic habitat with no physical barriers separating the two populations, which means that they exist in sympatry (Gerlach et al., 2001). By sequencing mitochondrial DNA (mtDNA) we were able to use a phylogeographic approach to evaluate two alternative hypothesis: (i) Lake Constance had been colonized by two genetically different perch populations from different refugia, which have come into secondary contact post-glacially. (ii) The two subpopulations reflect genetic differences that have developed in sympatry after the post-glacial colonization.

2.2 Materials and methods

2.2.1 Sampling, DNA extraction, amplification and sequencing

Eighty-two perch were analyzed for mtDNA D-loop sequence variation, corresponding to base 65 – 430 of the 5' end of the mtDNA control region in Eurasian perch (accession no. 14724). DNA samples of perch from Lake Constance and Lake Walensee were the same as in Gerlach et al. (2001), except for localities LA and OG where 3 and 7 new perch were caught. Additional perch were caught in the River Rhine, 17 upstream and 18 downstream of the waterfall (Fig. 2.1 (A)). Muscle tissue was stored in 80% ethanol until DNA extraction. Genomic DNA was extracted according to standard salt extraction procedures (Sambrook et al., 1989). For amplification of 365 base pairs of the mtDNA D-loop the primers HV2 and CSB-D (Nesbø et al., 1998a) were used under the published reaction conditions. PCR products were purified using the GFX PCR/DNA and Gel Band Purificationskit (Amersham Biosciences Europe GmbH, Freiburg, Germany). The same primers were also used as sequencing primers, whereby single-strand sequencing was carried

out for most individuals (using HV2 as sequencing primer). Double-strand sequencing was carried out for one individual of each haplotype (except haplotype T, using also CSB-D as sequencing primer). Sequencing was done by GATC (Biotech AG Konstanz, Germany), using an ABI 377 HT Automated Sequencer.

2.2.2 Data analysis

Alignment of mtDNA sequences was done by eye and a haplotype network was calculated using the computer program TCS (ver. 1.13, Clement et al., 2000). TCS uses a cladogram estimation method, also known as statistical parsimony described by Templeton et al. (1992) to estimate gene genealogies from DNA sequences that differ by only a few mutational steps.

For the analysis of geographical population subdivision we calculated pairwise F_{ST} estimates using conventional F-Statistics based on mtDNA haplotype frequencies with the software package ARLEQUIN (ver. 2.0 Schneider et al., 2000). Significance of the estimates was determined by a 10,000 step, 1000 iteration, Markov chain method.

2.3 Results

We found 10 different haplotypes based on 11 variable sites among Eurasian perch sampled at nine different localities (see Appendix for haplotypes and their distribution among localities). The two main haplotypes M (n= 53) and F (n= 15) were found in individuals from Lake Constance and the two Rhenian localities. The haplotypes M and F and one other haplotype F5 are identical to those described by Nesbø et al. (1999). In Lake Walensee and the two Rhenian localities sampled by Nesbø et al. (1999), the River Rhine and Lake Zürich, F was the sole haplotype found. The authors found the M-haplotype in the western part of the Danube but not in the Rhine, while the F-haplotype occurred in the southern part of the Rhine and in the Danube. The remaining eight haplotypes found in this study (F5 and M2 – M8) were very rare and appeared only in one individual (except haplotypes M2 and M4 which appeared in two individuals and haplotype M5 which appeared in 5 individuals). Haplotypes M2 – M8 seem to be unique to Lake Constance and the two nearby Rhenian localities and have not been found elsewhere in the European drainages sampled so far (Refseth et al., 1998; Nesbø et al., 1998b; Nesbø et al., 1999).

A haplotype network (Fig. 2.1 (A)) demonstrates the relationships between the different haplotypes. The two main haplotypes M and F are separated by four mu-

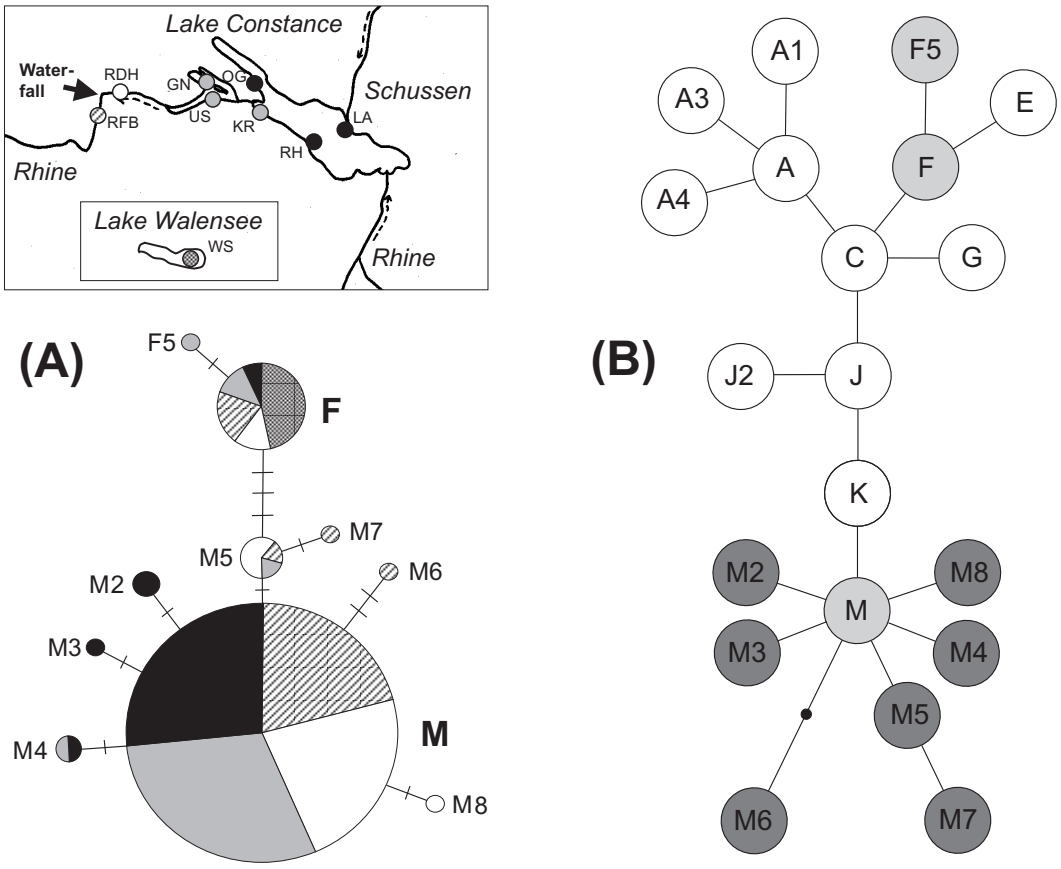

Figure 2.1: Haplotype networks from mtDNA sequence data of Eurasian perch generated by statistical parsimony as implemented by TCS (ver. 1.13, Clement *et al.* 2000). Haplotype designations refer to Nesbø *et al.* (1999) and this study. (A) Haplotype network for populations from Lake Constance, the two Rhenian localities and Lake Walensee. Black lines connect haplotypes, short bars reflect the amount of mutational steps between haplotypes. Circle sizes represent haplotype frequencies, and fill patterns refer to geographic localities shown in the map. (B) Haplotype network from mtDNA sequence data of the study area, including the main haplotypes for Eurasian perch from Nesbø *et al.* (1999). Black lines connect haplotypes and represent a single mutational step. Dark gray circles represent haplotypes found in this study, white circles represent haplotypes found by Nesbø *et al.* (1999). Light gray circles represent haplotypes found in both studies, small black circles represent missing haplotypes.

tational steps. Five of the seven newly found haplotypes differed from the common M-haplotype by one mutation, whereas M6 and M7 differed by two mutations.

If we include the main haplotypes found by Nesbø et al. (1999), all the missing nodes between haplotypes M and F are filled by other European populations. The newly found haplotypes all cluster with the M haplotype at the base of the haplotype cladogram (Fig. 2.1(B)).

MtDNA pairwise F_{ST}-values showed no significant differences between any of the six localities sampled within Lake Constance. When we combined the data of the three localities LA, OG and RH for subpopulation 1, and KR, GN and US for subpopulation 2, the two subpopulations did not differ significantly. Similarly, there were no significant differences between any of the Lake Constance and the two Rhenian localities (Table 2.1).

Table 2.1: Pairwise F_{ST}-values from mtDNA sequence data of Eurasian perch populations of Lake Constance (Population 1 and 2), the River Rhine (RFB, RDH, WS/ZS) and the western part of the River Danube (DN) calculated by ARLEQUIN. Significance values were determined by a 10,000 step, 1000 iteration, Markov chain method.

	Pop. 1	Pop. 2	RFB (dw)	RDH (uw)	WS/ZS† (R.lakes)
Population 1					
Population 2	-0.027				
RFB (dw)	-0.011	-0.026			
RDH (uw)	0.006	-0.014	-0.034		
WS/ZS† (R.lakes)	0.745***	0.746***	0.664***	0.679***	
DN†	0.231**	0.219**	0.121*	0.157**	0.398**

dw: downstream of the waterfall; uw: upstream of the waterfall; R.lakes: Rhine, lakes Walensee (WS) and Zürichsee (ZS).
* P<0.05; **P<0.01; ***P<0.001.
†: mtDNA data from Nesbø *et al.* (1999).

Thus, the population differentiation within Lake Constance, demonstrated by microsatellite analysis (Gerlach et al., 2001), could not be seen using mtDNA sequences. However based on our mtDNA data, the populations of Lake Constance and the two Rhenian localities were significantly different from the Lake Walensee/Lake Zürich population and from the population of the western part of the River Danube (Table 2.1).

2.4 Discussion

In contrast to the previous microsatellite analysis, showing that two subpopulations of Eurasian perch exist in sympatry in Lake Constance (Gerlach et al., 2001), no

evidence for a similar population split could be detected by pairwise F_{ST}-values of mtDNA haplotype sequences. If different mtDNA lineages have colonized the lake, the lack of differentiation today could be due to past hybridization and introgression. However, the two main haplotypes F and M might as well originate from colonization by two different mtDNA lineages, since the split between them is much older than the last glaciation (see Nesbø et al., 1999). To our opinion the existence of two genetically distinct subpopulations in Lake Constance has developed independently from the colonization of the lake by different mtDNA lineages and represents the result of sympatric differentiation. Similar conclusions were made by (Douglas et al., 1999) who examined models of evolution for morphologically different forms of *Coregonus* from the Central Alpine region, including three different forms from Lake Constance. Genetic diversity among populations of *Coregonus* was best explained by the species flock concept: repeated sympatric divergence of distinct populations, which occurred independently among these lakes, has lead to the evolution of multiple endemic forms.

In Lake Constance, the striking differences in morphology and associated differences in the trophic state of the two basins Upper Lake and Lower Lake could lead to differing selective forces acting on regional perch populations. Restricted gene flow due to philopatry, sexual selection or other behavioral mechanisms could then cause assortative mating, which will drive population splitting and may lead to sympatric speciation in the future (Dieckmann & Doebeli, 1999; Kondrashov & Kondrashov, 1999; Doebeli & Dieckmann, 2003).

Evidence for restricted gene flow and resulting population subdivision after colonization may also be seen at the mtDNA level since nearly all the derived haplotypes (M2, M3, M6, M7 and M8) were only found at individual localities whereas the ancestral M-haplotype was found at all localities. However the question remains, if the haplotypes derived from M (and F in one case) could have evolved during the very short time span of app. 15,000 years. Divergence rates of 6-7 % for the 5' end of the D-loop have been suggested for some perciform species like cichlids (Meyer et al., 1990). Based on these estimates, a divergence rate of 7% could be sufficient to allow for the evolution of the derived haplotypes within the lake.

The most frequent mtDNA haplotype in this study was the M-haplotype, which had mainly been found in the western part of the Danube (exceptions: 1 individual each in the rivers Tiber and Prut, Nesbø et al. 1999), where it co-occurs with the F-haplotype in a proportion of about 50% each haplotype. Within Lake Constance, 75% of all individuals were of the M-haplotype whereas only 3 individuals (7.5%) belonged to the F-haplotype (the remaining17.5% belonged to the derived haplotypes

M2-M8 and F5). Nesbø et al. (1999) hypothesized that the F-haplotype, which is 1% divergent from the M-haplotype, dispersed extensively in Western Europe and subsequently intergraded with the Danubian group. The strikingly different proportions of the two main haplotypes M and F in the Danube and within Lake Constance could possibly reinforce this hypothesis and date the time of colonization of the Danube by the F-haplotype to late or post Pleistocene. In our scenario, Eurasian perch from a Danubian source population colonized Lake Constance during the last glacial retreat. Two different scenarios could then explain the low number of F-haplotypes within Lake Constance: (i) both haplotypes colonized the lake from the Danubian drainage at the same time, and the F-haplotype suffered selective disadvantage that kept it at a low frequency. However, the F-haplotype found in the lower Rhine and the upper Danube seems to have expanded from a more northern (not Danubian) refugium (Nesbø et al. 1999). Thus during colonization of Lake Constance perhaps only the M-haplotype was available, so that (ii) a Danubian population containing exclusively M-haplotypes colonized Lake Constance. The occurrence of the F-haplotype within Lake Constance would then reflect a more recent secondary colonization event. Another piece of evidence for a secondary colonization of the lake by the F-haplotype could be found in the difference of the amount of derived haplotypes. The star phylogeny of the M-haplotype (Fig. 2.1) with seven derived haplotypes would seem to imply extreme demographic expansion and dominance, whereas only one derived F-haplotype was found.

On the other hand, colonization of Lake Constance by the F-haplotype originating from a Rhenian source population is unlikely but cannot be excluded completely. Ever since all the melt water from the late Pleistocene glacier drained into the River Rhine, the water had (and has still) to pass a huge waterfall (Fig. 2.2). This natural barrier probably precluded perch from the Rhine drainage to colonize Lake Constance. Even a highly migratory fish species like Atlantic salmon (S*almo salar* L.) was not able to overcome the waterfall and never did colonize Lake Constance.

Today, there is no connection between Lake Constance, which belongs to the Rhine drainage, and the Danube drainage. Thus the question remains how perch from the Danube could have colonized Lake Constance. During the Pleistocene glaciations, the entire region of the Alpine Rhine including Lake Constance was completely covered by the glacier (Fig. 2.2 (A-C)). Geological data show that during deglaciation huge temporal proglacial lakes, which were connected to the Danube drainage system, existed in front of the glacier (Keller & Krayss, 2000). These temporal lakes could have been used by perch to colonize Lake Constance. Fig. 2.2 (A-C) shows a likely scenario of colonization by M-haplotypes from the Danube

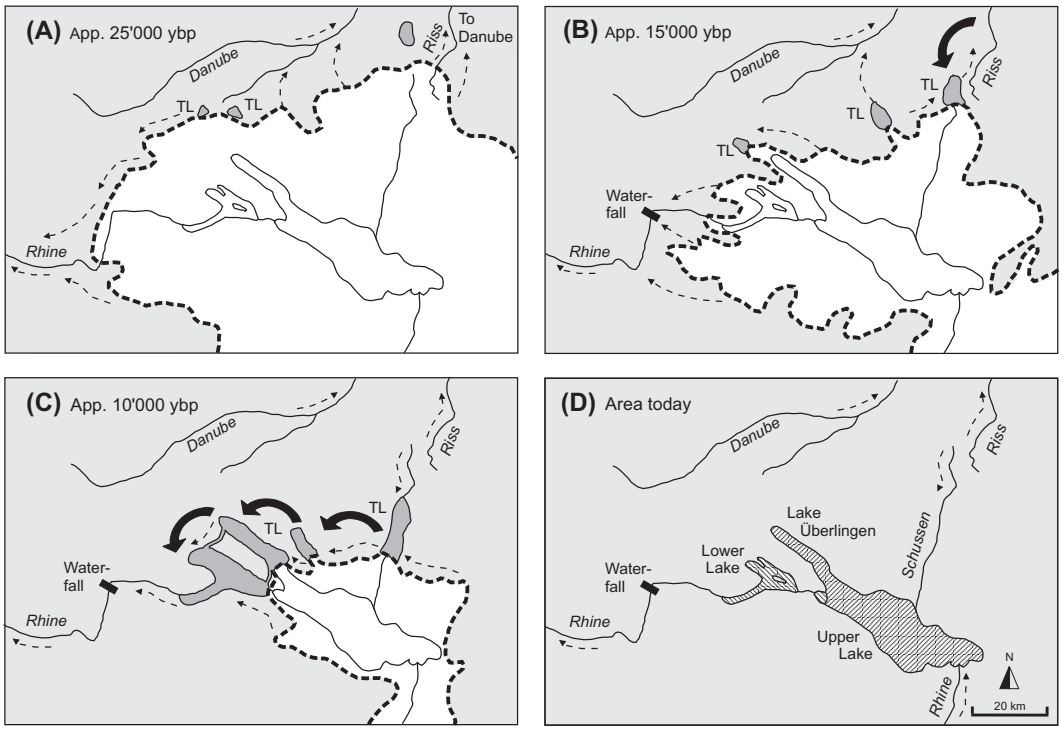

Figure 2.2: Map of the drainage area of Lake Constance, simplified from Keller & Krayss (2000) during and after the last (Würm) glaciation. Dashed arrows indicate directions of water flow. (A-C) shows the withdrawal of the glacier during three recessional stages. White area, glacier; TL, temporal proglacial lake; black arrows, hypothetical colonization route of Eurasian perch from the Danube system. (D) Lake Constance today. Hatched areas refer to subpopulations as revealed by microsatellite analysis (Gerlach *et al.* 2001). Forward slash, population 1, backward slash, population 2.

via one of these temporal lakes. As the glacier retreated, a drainage to the River Rhine became ice-free. Melt water from then on drained west to the Rhine, and the connection to the Danube system was cut off. Following the melt water streams, perch could have reached the first ice free basins of Lake Constance, Lake Überlingen and the Lower Lake which have never been completely isolated during the different recessional stages of the glacier withdrawal (Fig. 2.2(C)).

One question however remains unsolved, why the M-haplotype did not colonize more downstream parts of the River Rhine. In our study the M-haplotype was found in the same high proportion downstream of the waterfall as in Lake Constance itself (Table 2.2). This might be explained by a passive transport of fish within the water column. But not one individual of the M-haplotype was found in the downstream parts of the Rhine drainage system (Nesbø et al., 1999 and this study).

Table 2.2: Sampling sites of perch (as shown in Fig. 1) and mtDNA haplotype distribution, including two Rhenian and one Danubian population from Nesbø et al. (1999).

Population	Drainage	N	Haplotypes (n)
LA pop1*	Rhine (L.Constance)	7	F (1), M (4), M2 (2)
OG pop1*	Rhine (L.Constance)	6	M (6)
RH pop1*	Rhine (L.Constance)	6	M (4), M3 (1), M4 (1)
KR pop2*	Rhine (L.Constance)	8	F5 (1), M (6), M5 (1)
GN pop2*	Rhine (L.Constance)	6	F (1), M (5)
US pop2*	Rhine (L.Constance)	7	F (1), M (5), M4 (1)
RFB	Rhine (dw) ‡	18	F (3), M (12), M5 (1), M6 (1), M7 (1)
RDH	Rhine (uw) ‡	17	F (2), M (11), M5 (3), M8 (1)
WS	Rhine (L. Walensee)	7	F (7)
L. Zürich†	Rhine (L. Zürich)	10	F (10)
Rhine R. †	Rhine (France)	7	F (7)
Danube R.†	Danube (Austria)	20	F (11), M (9)

*: pop 1/2 refer to genetically distinct perch subpopulations in Lake Constance as shown by a microsatellite analysis with 5 loci (Gerlach *et al.* 2001).
†: mtDNA data from Nesbø *et al.* (1999).
‡: (dw), downstream of the waterfall; (uw), upstream of the waterfall.

Very similar results were obtained for brown trout (S*almo trutta* L.). Within the Danube drainage two mtDNA lineages, the Danubian and Atlantic lineage, were found (Osinov & Bernatchez, 1996; Bernatchez, 2001; Weiss et al., 2001), whereas in the Rhine drainage only the Atlantic lineage occurred (Osinov & Bernatchez, 1996; Bernatchez, 2001). For perch in the Rhine system, no physical barriers existed since the last glaciation that could prevent the M-haplotype from expanding within the Rhine, once it is flushed down the waterfall. We suggest, following the arguments of Bernatchez (2001) for brown trout, that partial genetic incompatibilities of the haplotype lineages could have accumulated during their geographical isolation, which then limited introgressive hybridization between them. Further, the M-haplotype as the later migrant may have been unable to colonize habitats where the F-haplotype as the pioneer disperser was already established. Both factors together may then have prevented the M-haplotype from colonizing the Rhine drainage system, a hypothesis that needs further testing.

Chapter 3

'To whom do I belong?' Population and Kin Recognition as mechanisms for shoal formation leading to population divergence in Eurasian Perch (*Perca fluviatilis* L.)

Prior studies on fish shoals have shown that they often consist of individuals, matched in size or age or that are closely related. The gene frequencies of five microsatellite loci were used to test, if larval perch (*Perca fluviatilis* L.) form aggregations of closely related individuals during their highly mobile pelagic phase. The analysis revealed that 3 out of 5 larval aggregations tested showed significant accumulation of related individuals. To investigate the underlying mechanisms of shoal formation, preference tests were conducted in a flume. Two different odor stimuli were presented simultaneously on two sides of the water current. A group of four test fish was allowed to choose between odor of either unfamiliar full-sibs or unfamiliar half-sibs versus the odor of unfamiliar non-sibs of the same subpopulation. The same experimental set-up was used to test for population recognition, presenting odor of unfamiliar non-siblings of the same subpopulation versus odor of unfamiliar conspecifics of the foreign subpopulation. The behavioral experiments showed that perch significantly preferred kin over non-kin and members of the same over the foreign subpopulation, independent of familiarity. Thus we are able to present a behavioral mechanism that could lead to the observed formation of kin structured shoals in perch. We discuss possible advantageous in terms of altruistic interactions due to kin preference and in terms of speciation processes due to possible assortative mating within subpopulations.

3.1 Introduction

Shoaling in fish is a very common phenomenon and can be found throughout numerous freshwater and marine species. Shoaling fish gain benefits by predator evasion, predator confusion, the dilution effect and other antipredator functions (Godin, 1986; Magurran, 1990; Pitcher & Parrish, 1993). Members of shoals can also optimize their foraging efficiency in finding food faster (Pitcher et al., 1982; Street & Hart, 1985), and spending more time feeding (Magurran & Pitcher, 1983). Studies on the function of fish shoals no longer invoke altruism to account for group-wide coordination but have shown that shoal members act as selfish individuals. Especially under predator attack it is usually in the interest of individuals to coordinate their behavior (Pitcher & Parrish, 1993). However, there still exist enigmatic behaviors like the risky predator inspection behavior (Milinski, 1987) or the release of fright substance (*Schreckstoff*) (Smith, 1986). These findings together with investigations on kin recognition (Brown & Brown, 1992,1993; Arnold, 2000) and kin related genetic structuring of fish shoals (Ferguson & Noakes, 1981; Pouyaud et al., 1999; Gerlach et al., 2001) could be seen as evidence that in some fish, altruistic shoal behavior may have evolved through kin selection (Smith, 1982; Pitcher & Parrish, 1993). The background would be that closely related animals are more likely to show co-operative behavior during risky situations because helping a relative can increase the indirect fitness of an individual (Hamilton, 1964).

Nevertheless living with relatives can also have disadvantages through the risk of inbreeding which increases the genetic homozygosity and thus the possible expression of recessive deleterious mutations in offspring (Charlesworth & Charlesworth, 1987). Kin recognition mechanisms can be used to identify related conspecifics to shoal with but also to avoid closely related breeding partners. It can therefore be advantageous in different directions. An individual may profit from the benefits of co-operation with relatives and on the other hand can minimize inbreeding depression by avoiding to mate with siblings.

This study focuses on Eurasian perch (*Perca fluviatilis* L.), a very common freshwater fish species with a wide distribution in a variety of different habitats in the northern temperate hemisphere (Thorpe, 1977). A previous study on the genetic relatedness of perch using five DNA-microsatellite markers showed that two genetically distinct sub-populations exist within Lake Constance ($G_{ST} = 0.07$). In Lake Constance, one population occurs in the eastern, the other in the western part of the lake. No obvious physical barriers separate the two coexisting populations. A similar stock structure could also been shown for Eurasian perch in Lake Windermere (Kipling & Le Cren, 1984; Bodaly et al., 1989) and for yellow perch in Lake Michi-

gan (Miller, 2003). For Lake Constance perch, pairwise calculations of relatedness provided evidence that shoals of juvenile and adult perch in the littoral were genetically structured within age groups consisting mainly of closely related kin (Gerlach et al., 2001).

The life history of perch provides evidence that the genetic structuring within this species could be due to a preference to shoal with distinct conspecifics like closely related or familiar fish. Perch as well as its sister species yellow perch (*Perca flavescens* L.) spawn in the shallow littoral zone in early spring. Females lay their eggs in one long egg-strand. Soon after hatching perch larvae move to the pelagic zone were they feed on zooplankton. After about one month they return to the littoral and switch to benthic prey (Melbourne et al., 1985; Wang & Eckmann, 1994). The kin structure found in shoals of juveniles and adults could either be the result of homing of perch to their natal sites, or it is the result of a preference to shoal with particular fish. These could be familiar fish that simply hatched nearby at the same time and are therefore very likely to originate out of the same egg-strand and thus siblings.

A preference to shoal with familiar fish has been shown for a variety of freshwater fish like guppies (Griffiths & Magurran, 1999), bluegill sunfish (Brown & Colgan, 1986; Dugatkin & Wilson, 1992) sticklebacks (Van Havre & Fitzgerald, 1988) and brown trout (Höjesjö et al., 1998). On the other hand kin structure could be due to kin recognition and a preference to shoal with close relatives. Kin recognition was found for some salmonid species like Arctic charr (*Salvelinus alpinus*) (Olsén, 1989), Atlantic salmon (S*almo trutta*) (Brown & Brown, 1992) and rainbow trout (*Oncorhynchus mykiss*) (Brown & Brown, 1992) and for rainbowfish (*Melanotaenia eachamensis*) (Arnold, 2000). Contradictory results were found for coho salmon (*Oncorhynchus kisutch)* (Quinn & Busack, 1985) but see (Quinn & Hara, 1986), for sticklebacks (*Gasterosteus aculeatus*) (Van Havre & Fitzgerald, 1988; Frommen & Bakker Theo, 2004) but see (Steck et al., 1999) and for the Trinidadian guppy (*Poecilia reticualta*) (Warburton & Lees, 1996) but see (Griffiths & Magurran, 1999). Some authors investigated the genetic structure of fish shoals in the field using allozyme markers and recently microsatellites (Ferguson & Noakes, 1981; Avise & Shapiro, 1986; Dowling & Moore, 1986; Naish et al., 1993; Hauser et al., 1998; Pouyaud et al., 1999). Kin structure could so far only be found for shoals of the mouthbrooding tilapia (*Sarotherodon melanotheron*) (Pouyaud et al., 1999).

Unlike kin recognition, olfactory based population recognition in fish has not been investigated very often. To our knowledge, the only studies have been done for salmonids (for a review see Olsén, 1992). Evidence for population recognition

based on chemical cues was given for Atlantic salmon (*Salmo salar*) (Stabell, 1982; Stabell, 1987), for Arctic charr (Olsén, 1986) and for coho salmon (Courtenay et al., 1997).

Olfactory recognition and preference in fish could be based on phenotype matching, learned from related individuals (Brown et al., 1993; Tang-Martinez, 2001). Variability of MHC alleles is probably involved in these recognition processes. Female sticklebacks differentiated between males according to their MHC alleles (Reusch et al., 2001); juvenile Arctic charr chose the water scented by fish with their own MHC type (Olsén et al., 1998, 2002).

The aim of our study was to investigate, whether kin aggregations like those observed in juvenile and adult perch, are already present in free ranging shoals of larval perch. Preference tests in the laboratory should show, whether the genetically structured shoal formation in perch might be based on kin recognition and maybe kin selection and is therefore independent of familiarity. Additionally, we investigated, whether perch can distinguish between conspecifics of the same versus the foreign subpopulation.

3.2 Materials and methods

3.2.1 Microsatellite genotyping

Sampling, DNA extraction, microsatellite typing

Previous investigations by (Wang & Eckmann, 1994; Wang & Appenzeller, 1998) have shown that soon after hatching in the littoral, perch larvae can be found in the pelagic zone of the lake were they are distributed mainly near the surface. Based on this knowledge, larvae were sampled with horizontical trawls of a plankton net near the surface in the pelagic zone of Lower Lake Constance (western population). The plankton net had an opening of 0.25 m^2. Five conical nets could be opened and closed individually one after the other. We conducted five consecutive samples. One net sampled along 140m length. The speed of the boat was approx. 0.6 m s^{-1}.

Total genomic DNA from 20 larvae per sample (19 larvae in sample 5) was extracted according to standard salt extraction procedures (Sambrook et al., 1989). Larvae were genotyped using five dinucleotide microsatellite loci comprising three isolated from walleye (*Stizostedion vitreum,* loci SVI 6, 17, 18; GenBank Accession no.: G36962; -63; -64, Borer et al., 1999) and two from yellow perch *(Perca flavescens,* loci PF 1,5; GenBank Accession no.: AF211826; -30, Leclerc et al., 2000). The Polymerase Chain Reaction (PCR) was conducted in 10 μl reaction volumes using standard conditions. A summary of the loci characteristics is presented in Table

3.2. The PCR products were run individually on Spreadex gels (Spreadex^{TM} gels, Elchrom scientific AG, Switzerland: EL 400 for SVI 6,17 and PF 1,5; EL 600 for SVI 18) using the SEA 2000^{TM} advanced submerged gel electrophoresis apparatus (Elchrom scientific AG, Switzerland). Gels were run at 120 V, 990 mA. Running time depended on allele sizes. Alleles were visualized by dyeing with sybr gold. The allelic size of the PCR products was determined by comparison with a standard M3 marker ladder (Elchrom scientific AG, Switzerland).

Table 3.1: Towing distance and number of larval perch caught with plankton nets in the pelagic zone of Lower Lake Constance.

Sample no.	1	2	3	4	5
Towed distance [m]	163	128	148	144	139
No of fish	43	103	131	69	58

Data analysis

Observed and expected values for heterozygosity were determined using the GENETIX 404 computer package (Belkhir et al., 1997). For calculations of genetic relatedness, the pairwise identity index I_{xy} (Mathieu et al., 1990; Castric et al., 2002) was estimated using the IDENTIX computer package (Belkhir et al., 2002), which can detect relatedness in populations using multilocus genotypic data. Closely related individuals are more likely to share alleles for distinct loci and therefore produce homozygous offspring. To calculate the identity index, first the proportion of homozygous alleles in the putative offspring of every pair of individuals was calculated. To estimate, if the individuals in the population are more closely related than would be expected if the parents had mated randomly, a so called null distribution of random mating was calculated by the program. This null distribution was obtained by a resampling procedure without replacement. 2N alleles were selected independently for each locus, assigning them at random from the allele distribution of the N individuals. After this procedure, the statistics were recalculated. The null hypothesis of random mating parents was rejected, if the observed value of the statistics was above the 95% level of the resampled statistics. In our case, the method had to be extended, because our aim was to detect relatedness within distinct groups (aggregations of perch) out of the same population. The method was the following: the identity index for each of the five net samples was calculated. To test if larvae within one sample were genetically more related than randomly expected, the mean identity index of all perch pairs within one sample was tested against the null distribution

of no relatedness. For calculating the null distribution, we used the distribution of identity indices of randomly generated sub-samples of the same sample size (20 individuals). Sub-samples were generated by random permutation of genotypes in 1000 randomized sub-samples using all five samples as genotype pool. The statistic procedure of testing for significance was then the same as described above.

3.2.2 Behavioral experiments, kin- and population recognition.

Test and stimulus fish

During spawning time in May 2001, perch were captured with gill nets from Lake Constance, Germany. To obtain full sibs, the egg-strand of a ripe female was artificially fertilized with the sperm of one ripe male. To obtain groups of half sibs we used the following procedure: For paternal half sibs, the same male was used to fertilize pieces of egg-strands of different females. For maternal half sibs the egg-strand of one female was cut into pieces, which were then fertilized each by a different male. All egg-strands or pieces of egg-strands were transported to the laboratory and placed separately in 9 l aquaria (constant supply of tap water, 0.1 l min^{-1}, temperature 15 $^{\circ}$C, 14 h illumination with overhead fluorescent tubes). Thus, each aquarium contained a group of full-sibs. All groups were visually separated by placing opaque screens between the aquaria. After hatching of larvae temperature was raised to 20 $^{\circ}$C during the first week. Perch larvae were fed with copepod nauplii and rotifers for the first 4 days, and afterwards with live artemia nauplii and daphnids. Juveniles were fed with frozen chironomids. Preference tests in a flume were carried out with fish between 3 and 6 months of age.

Test flume

Perch were tested in a flume (200 cm x 25 cm, water level 9 cm, Fig. 3.1) built of grey PVC. A similar construction was used by (Höglund, 1961; Steck et al., 1999; Atema et al., 2002) for experiments on the response to chemical gradients and on kin discrimination in fish. The flume was divided into three compartments, the inlet compartment, the test area and the outlet compartment. The inlet compartment was separated in two equal halves by a PVC wall (100 cm long) presenting two bodies of moving water separated by a sharp boundary in the test area. Evenness of water flow on both sides of the test compartment was visualized using fluorescent dye. The test area (33 cm x 25 cm) was separated by screens from the other compartments preventing fish from entering the two arms of the inlet area or the outlet compartment. A slow but permanent flow-through of tap water (4050 ml min^{-1}, velocity 0.6 cm s^{-1}, temperature 20 $^{\circ}$ C) ensured that no chemical information about perch was initially present in the flume or was accumulating in the system during

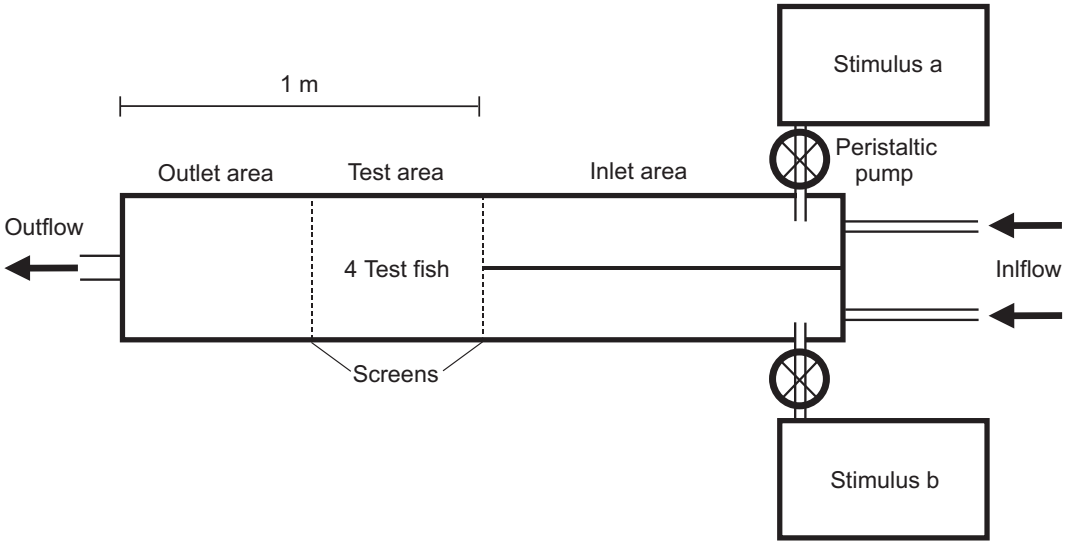

Figure 3.1: Experimental flume for preference tests with juvenile perch. For details see text.

the trials. To obtain stimulus water, one liter of water was taken from the holding tank of the respective stimulus group and used immediately. Stimulus water (7.6 ml min^{-1}) was added to either side of the inlet compartment using a peristaltic pump (Ismatec, MCP V5.16). The test area was visually isolated by black cloth from all sides. The compartment was illuminated by two halogen bulbs (same light cycle as in the holding tank). All observations were video taped for later analysis.

Procedure

In preliminary trials we tried to use a single perch for the preference tests. Unfortunately, one single fish showed erratic behavior and no food intake. Therefore, we decided to use a small group of full sibs out of the same holding tank as test fish.

Four fish of one sibling group were placed in the test area. Acclimation time was between one and eight days, during which fish were fed daily with frozen chironomids. For one test group all preference tests (trials) were carried out on the same day between 10:00 a.m. and 04:00 p. m. Four different trials were run, with consecutive trials separated by at least 30 min recovery time, when no odor stimulus was supplied. The trial began by starting the video recorder. After three min without odor application, stimulus water was supplied for five min. After odor supply started we waited for two minutes (odor plume needs one min. to reach the downstream end of the test area) before we started counting the number of fish present on either halve of the test area every five seconds for three consecutive minutes. After that time, odor supply was stopped for three min and the sides of stimulus supply were

changed. This procedure was repeated until the odor stimulus had been presented twice from each side. After the last trial all four test fish were removed from the flume and excluded from further experiments, including the production of stimulus water. However, most test fish were used for producing stimulus water for other test groups before they were used as test fish themselves.

The following trials were run with the order of trials set at random; 1) full-sibs vs. non-kin, 2) maternal half-sibs vs. non-kin, 3) paternal half-sibs vs. non-kin, and 4) western and eastern population.

3.2.3 Data analysis

For each trial, we calculated the average number of test fish present on that side of the flume, where a particular stimulus was provided. One-way ANOVA was used to test for differences between the different kin recognition trials (full-sibs, maternal or paternal half-sibs versus non-kin). Since there were no significant differences, the data was pooled for further analysis. A Wilcoxon signed rank test was used to compare the average number of fish on each of the two stimulus sides (kin versus non-kin or same versus foreign population). Hereby the different groups of test fish were considered as replicates.

Sometimes groups of test fish, although swimming actively, never changed sides during one supply of stimulus water. Therefore they were not able to make a choice between odor stimuli. Additionally, sometimes test fish were completely inactive (fish were resting at the bottom) and thus also not able to choose between different stimuli. To eliminate such time intervals we calculated a so-called "activity index" for each of the three min of odor supply. We divided the observed amount of changes between the two sides of the test area during one min of odor supply by the maximal amount of side changes possible for four fish during one min of odor supply (4 x 12 five sec intervals = 48 side changes possible). If for example 19 side changes were observed for the first min of odor supply the activity index would be 19 / 48 = 0.4, i.e. 40 % of the max. amount of side changes possible. We eliminated minutes with activity indices lower then 0.1 (10% of max. amount of side changes possible). This method is very conservative because in cases when fish change back and forth between consecutive observations or where two fishes just change sides, we did not count a side change. Thus, using this method we underestimated the amount of side changes and therefore maybe lost some data for statistical analysis.

3.3 Results

3.3.1 Microsatellite genotyping, genetic structure of pelagic shoals of perch larvae

We caught 404 perch larvae within the pelagic zone of Lower Lake Constance (Table 3.1). Twenty larvae from each sample were genotyped, using five microsatellite loci. The degree of polymorphism at five microsatellite loci varied between 5 and 12 alleles per locus. Observed levels of heterozygosity were moderate and ranged from 0.404 to 0.687 (Table 3.2).

Table 3.2: Microsatellite characteristics. Perch larvae (N: 99) examined with five microsatellites were caught in the pelagic zone of Lower Lake Constance (western population). Subscripts at the locus names show the repeat motives. H_{obs} =observed heterozygosity, H_{exp} = unbiased expected heterozygosity (Nei, 1978).

Locus	No. of alleles	Size range [bp]	H_{obs}	H_{exp}	Primer sequences
SVI $18_{(AC)}$	6	160-174	0.616	0.546	GATCTGTAAACTCCAGCGTG
					CTTAAGCTGCTCAGCATCCAGG
SVI $17_{(AC)}$	6	110-142	0.475	0.550	GCGCACTCTCGCATAGGCCCTG
					CGTTAAAGTCCTTGGAAACC
SVI $6_{(AC)}$	5	106-120	0.667	0.616	CATATTATGTAGAGTGCAGACCC
					TGAGCTTCACCTCATATTCC
PF $1_{(GA)}$	12	112-140	0.687	0.737	AAGCAGCCTGATTATATATC
					CAGACAATTAAACATGCAAC
PF $5_{(GT)}$	6	134-152	0.404	0.471	TGAGAGCCCATGAATTAC
					GCAAACACAGCCAATTTAG

The I_{xy} values for the putative offspring of randomly generated pairs (subsamples of 20 individuals) is shown as null distribution in Fig. 3.2, together with the mean I_{xy}'s for each sample. The mean pairwise identity index in three of the five net samples departed significantly from the null expectation of no relatedness (Fig. 3.2, two tailed test, sample 1: p=0.01; sample 2: p=0.20; sample 3: p=0.35; sample 4: p=0.01; sample 5: p=0.04).

3.3.2 Behavioral experiments

Eurasian perch showed a clear reaction to odor supply of stimulus water from conspecifics. Before odor supply started, all four test fish were often inactive, lingering together at one side of the flume. Approx. one to two minutes after the beginning

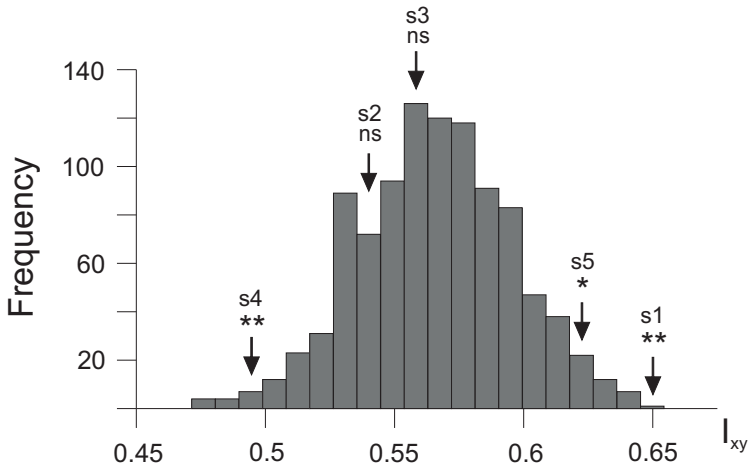

Figure 3.2: Distribution of relatedness coefficients (pairwise identity index calculated by the IDENTIX computer package of Belkhir, 2002). The distribution was calculated for a sub-sample of perch larvae, caught in the pelagic zone of Lake Constance (for details see text), assuming random association of monolocus genotypes. Arrows indicate observed values of five samples (1-5) of perch larvae.

of odor supply (odor plume needs one min. to reach the downstream end of the test area) fish became active. They swam upstream and pressed their snouts against the screen trying to enter the arms of the inlet area. This so called "screen-swimming" was also observed in kin discrimination tests with cichlids and in population recognition tests with salmonids (Barnett, 1986; Courtenay et al., 1997), and was used as a measure for side preference.

Stimulus perch in our kin recognition tests were differently related to test fish. They could be either full-sibs, maternal half-sibs, paternal half sibs or unrelated. Given that no significant difference in preference could be seen between the three different kin recognition tests (ANOVA, among all categories of different sibships $F_{2,30} = 2.06$, $p = 0.146$), all kin data were pooled. Perch showed a significant preference for their siblings versus non-kin (paired t-test, $t = 3.43$, $n = 33$, $p = 0.002$; Fig. 3.3(A)).

Given the choice between fish of the same versus the foreign population, perch significantly preferred conspecifics of the same subpopulation (paired t-test, $t = 2.36$, $n = 13$, $p = 0.04$, Fig. 3.3(B)).

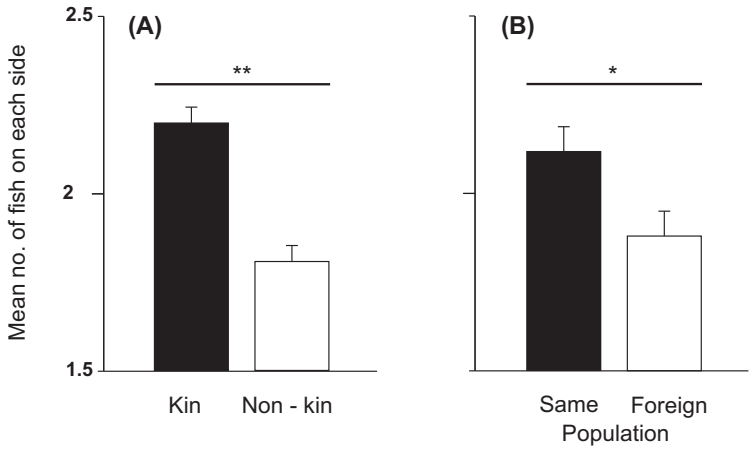

Figure 3.3: Olfactory preference of perch, tested in a flume. (A) Black bar, side of the flume, where odor stimulus of kin was presented, light bar, side of the flume, where odor stimulus of non-kin was presented (paired t- test, $t = 3.43$, $n = 33$, $p = 0.002$). (B) Black bar, side of the flume, where odor stimulus of the same population was presented, light bar, side of the flume, where odor stimulus of the foreign population was presented (paired t- test, $t = 2.36$, $n = 13$, $p = 0.04$)

3.4 Discussion

The genetic analysis showed that larval perch already form kin structured shoals. In laboratory experiments juvenile perch differentiated between kin and non kin and between members of the own and the foreign subpopulation.

3.4.1 Kin structure of larvae aggregations

Two out of five larvae samples that were collected in close proximity shared more and one sample shared less alleles than randomly expected, both indicating relatedness. This seems to be puzzling but the level of relatedness in a family group highly depends upon the specific genotype of the parents. If some of the shoals consisted of separate families that happened "by chance" to be less related than any two families taken randomly from the population, relatedness would be significantly lower (Bernatchez and Castric personnel communication). Our sampling method did not allow the identification of distinct larval shoals. Thus a sampling of two or more shoals and also of different families within one trawl could well have occurred. Given the conservative statistical analysis (Castric et al, 2002) we assume that in reality the degree of relatedness in aggregations of perch larvae might be even higher. These kin groups may persist for years because a high degree of relatedness was found in groups of juvenile and adult perch (year-classes 0 to 2, Gerlach, 2001).

3.4.2 Kin recognition

We could show that a fish species, which has been found in kin aggregations in the field (Gerlach et al., 2001, and this study), recognizes and prefers chemical stimuli of siblings in laboratory experiments. Until recently the rather few studies of this type have shown contradictory results. Brown & Brown (1992; 1993; and 1996) have shown in numerous laboratory experiments that salmonids including Atlantic salmon can discriminate kin and show kin-biased social behavior by gaining benefits in higher growth rates and lesser aggressive interactions with siblings as territory neighbors. In a field study, (Fontaine & Dodson, 1999) investigated the relationship of Atlantic salmon larvae and fry in their natural habitat in order to test the hypothesis that closely related fish should occupy neighboring territories to gain benefits of kin-biased interactions. Although numerous pairs of fish were closely related as shown by microsatellite analysis, related fish did not preferentially occupy neighboring territories.

Studies on threespined sticklebacks show even more conflicting results. Whereas Van Havre & Fitzgerald (1988) could show stickleback fry to preferentially shoal with kin when exposed to optical and chemical stimuli in the laboratory, Steck et al. (1999) found no preference for siblings in juvenile sticklebacks using only chemical stimuli in a flume. However, a recent analysis with adult sticklebacks revealed preference for familiar kin (Frommen & Bakker, 2004). Genetic data on allozyme polymorphism in 24 free ranging stickleback shoals did not show any kin structure within fish shoals (Peuhkuri & Seppae, 1998).

If aggregations of newly hatched perch are transported passively to the pelagic zone due to water currents related fish would stay together independent of any preference for distinct conspecifics. From the observation of shoals, we have some indications that perch larvae support the passive transport by active swimming movements. However, during the pelagic phase perch grow to free-ranging, active swimmers and obviously will meet other shoals of unrelated fish. Meeting shoals will have to decide whether to stay together with familiar fish or mix and form new shoals. Our genetic analysis on the relatedness of perch larvae clearly shows that even during the mobile pelagic phase kin structure of shoals is maintained. So far, the kin structure in perch shoals could still be the result of a preference to shoal with familiar fish. The behavioral experiments made in this study show a preference for kin independent of familiarity, since pieces of egg-strands for the production of half sibs had been separated before fertilization. Additionally paternal half-sibs also showed a tendency to prefer kin over non-kin (One tailed t- test, $Z = 1.57$, $n = 9$, $p = 0.08$) indicating that maternal cues are not necessary for recognition. Our

results suggest that kin recognition in perch could be based on phenotype matching. Probably differences in MHC alleles are involved in the recognition mechanism.

Juvenile perch (especially young of the year perch) are a preferred prey item of many piscivorous fish and avian predators (Gliwicz & Jachner, 1992; Diehl, 1995). Shoal formation could therefore primarily be due to anti predator and maybe optimal foraging functions. If shoaling with relatives is advantageous in terms of altruistic interactions like i. e. predator inspection it would be a very interesting topic for further studies on adaptive behaviors in perch. Gerlach et al. (2001) could show that adult perch could be found in kin structured aggregations as well. Adult perch in Lake Constance mostly lack being preyed upon except maybe by a few big pike or pikeperch. Therefore, shoaling with kin could be beneficial in a different way, i.e. by the performance of a piscivorous group foraging strategy with its efficiency depending on mutualistic interactions (Eklöv, 1992).

Additionally, the ability to recognize kin could be helpful in finding the preferred shoaling partners after solitary resting phases. Helfman (1979) investigated the twilight activity of yellow perch by scuba diving. With decreasing light intensity after sunset, activity of perch slows down, shoal formation breaks up and fish settle down for the night. In the morning the reversed behavioral patterns were observed. Fish began to move and slowly formed shoals again. A similar solitary resting behavior at night could be seen for Eurasian perch in Lake Constance (Fischer, personal information).

3.4.3 Population recognition

In this study we could show for two sympatric perch populations that juveniles of one population could recognize the own versus the foreign population by waterborne chemical cues. Most experiments on population recognition have been done with salmonids. Migrating salmonid species learn about the odor of their natal rivers as juveniles and use it for orientation when they return from the sea as adults to spawn in freshwater rivers (reviewed in Hasler & Scholz, 1983). However it has also been hypothesized that salmonids are guided to their natal rivers by population specific odors of juveniles. Preference tests in the laboratory showed that salmonids can recognize population specific cues and mostly prefer the odor of the same over a foreign population (reviewed in Olsén, 1992). The preference for population specific odors seems to be independent of familiarity (Courtenay et al., 1997).

We could show that juvenile perch prefer the own versus a foreign subpopulation that lives in sympatry within the same lake. If this preference also holds for the mate choice of adult perch, it could be a reason for the maintenance of popula-

tion structure by assortative mating with members of the own subpopulation. This would be a very interesting topic for further studies on speciation in perch. Numerous investigations on speciation have been done on the threespine stickleback. The threespine stickleback can often be found in two sympatric forms, a benthic and a limnetic form (McPhail, 1984, 1992; Schluter & McPhail, 1992). The two forms are reproductively isolated by assortative mating whereby interspecific mate preferences seem to be dominated by body size and to some extent also by behavioral differences between the two forms (Ridgway & McPhail, 1984; Nagel & Schluter, 1998; Hatfield & Schluter, 1996). Additional reinforcement of population subdivision in sticklebacks could be the reduced fitness of hybrids, which seems not to be caused by a hybrid mating disadvantage but by ecological speciation of the two sympatric forms, and eventually by direct selection on hybrids in the field (Hatfield & Schluter, 1996, 1999). Focusing on studies about morphological differences between subpopulations of perch and investigations about hybrid viability and fitness of hybrids we want to learn more about population subdivision in perch.

3.5 Conclusion

With the behavioral experiments, we present a possible mechanism for the observed kin aggregations, since perch are able to differentiate and prefer full and half-sibs by the use of olfactory cues. Additionally, we could show that perch can differentiate between their own and the foreign subpopulation. This behavior could lead to assortative mating and to ongoing population subdivision. We conclude that population coherence can be an active choice based on olfactory preference. We did not yet analyze mating preferences, but the genetic differences between the two populations suggest limited gene flow between them.

Chapter 4

Unconditional isolation between two sympatric populations of Eurasian perch (*Perca fluviatilis L.*)

To test mechanisms for reproductive isolation between two sympatric perch populations, we conducted laboratory experiments on hybrid fitness. Fertilization rate and hatching of parental populations were compared with F_1 hybrids between populations. Hybrid Fitness was significantly reduced indicating intrinsic genetic incompatibility between the two subpopulations. Our results suggest that population divergence in perch may be reinforced by reduced hybrid fitness, i.e. by unconditional isolation.

4.1 Introduction

Evolutionary biology tries to understand the mechanisms that cause speciation and lead to species diversity on earth. It is generally accepted that speciation occurs as a by product of factors like divergent natural and sexual selection, genetic drift, founder events and polyploidization (Mayr, 1963; Futuyma, 1998), whereby reproductive isolation is the ultimate cause for speciation (Mayr, 1942; Barton & Charlesworth, 1984; Coyne, 1992). Hybridization between populations can act contra dictionary to divergence, since it results in a mixture of already discrete gene pools. However, hybridization and introgression can also increase genetic diversity and can result in new recombinant species as has been shown for some plant (Barton, 2001) but also for some animal taxa (Dowling & Secor, 1997). It is generally accepted that strict allopatry can lead to speciation (Mayr, 1963). However, it remains unclear, how complete the cessation of gene flow between allopatric populations may be, especially if they come into secondary contact. Evolutionary

mechanisms like prezygotic isolation (mostly mate choice behaviors like assortative mating) and postzygotic isolation (reduced viability or fertility of hybrids) can reduce hybrid offspring (reviewed in Rice & Hostert, 1993). Due to the high costs of hybridization, Dobzhansky considered already in 1940 the evolution of mechanisms that convert postzygotic to prezygotic isolation. This process is known as secondary reinforcement (Dobzhansky, 1940). Evidence for reinforcement could be found in the extended studies on sympatric *Drosophila* species (Coyne & Orr, 1989, 1997) and in sticklebacks (Vamosi & Schluter, 1999).

New theoretical models provide evidence that speciation can also occur in sympatric populations (Tregenza & Butlin, 1999; Turelli, 2001). Differential selection on ecotypes associated with assortative mating can lead to disruptive selection despite the presence of gene flow (Kondrashov & Shpak, 1998; Dieckmann & Doebeli, 1999; Kondrashov & Kondrashov, 1999; Kirkpatrick, 2000; Doebeli & Dieckmann, 2003). An important constraint on disruptive selection can be selection against hybrids. (Rice & Hostert, 1993) showed that hybrids can suffer reduced fitness, both because they may fall between distinct ecological niches of their parent populations and are selected against ("environment-dependant postzygotic isolation"), and/or because of intrinsic genetic incompatibilities between the parental populations ("unconditional isolation"). Schluter and colleagues tested whether environment-dependant and/or unconditional isolation play a role in stickleback speciation. They could show that hybrids of a sympatric species pair, a benthic and a limnetic form, had lower growth rates in both parental habitats. This was regarded as an indication for environment-dependant postzygotic isolation. However only slight evidence for unconditional isolation was found (Schluter, 1995; Hatfield & Schluter, 1999).

Our study focuses on Eurasian perch (*Perca fluviatilis* L.). In Lake Constance, two genetically different perch populations ($G_{ST} = 0.07$) coexist. One population inhabits the eastern the other the western part of the lake. Since no geographical barrier separates the populations, they live in sympatry (Gerlach et al., 2001). In the shallower Lower Lake Constance (area of western population) water temperature increases earlier in spring, so the western populations spawns approx. two weeks earlier than the eastern population. The whole spawning time lasts for about one month but Lake Constance perch show a so called "mass spawning" behavior where most of the fish reproduce within one week (Wang & Eckmann, 1994).

The aim of our study was to investigate mechanisms that might reinforce reproductive isolation and drive population divergence in Eurasian perch. In a first approach, we tested F_1 hybrid crosses of both populations for two fitness parameters, fertilization rate and hatching success, to investigate whether unconditional

isolation plays a role in population divergence of these sympatric perch populations.

4.2 Materials and methods

During the main spawning time of the western population, gill nets were exposed over night in the areas of both populations. Early next morning, males of the eastern population were carefully removed from the gill nets and transported alive to the area of the western population. As soon as a ripe female of the western population was removed from a gill net, her egg-strand was stripped and immediately fertilized with the sperm of a male from the eastern population. Ripe females released their entire egg-strand upon slight pressure on the abdomen. If females were not completely ripe, the egg-strand was released in pieces and under high pressure on the abdomen. For our experiments, only undamaged egg-strands from ripe females were used. Adult males by contrast released high amounts of sperm by a slight pressure on the abdomen during the entire spawning period. For females of the eastern population we conducted the same artificial fertilization procedure vice versa. For parental population crosses, males and females of the same area were used. Four types of crosses were made, using each fish only once for egg or sperm production. Parental crosses: eastern population (N = 5); western population (N = 6); Hybrid crosses: $F_1$1, mother eastern population / father western population (N = 7) and $F_1$2, mother western population / father eastern population (N = 9).

We were not successful to catch enough ripe females from the western population to conduct hybrid crosses $F_1$2 . Therefore, we took seven females that were not yet ripe to the laboratory. They were kept in a 250 liter aquarium (10 °C, permanent flow-through of lake water). Since females may become ripe and running within a few hours, we checked the females twice a day applying slight pressure on the abdomen. As soon a female could be stripped (for 3 females on the next day, for 3 other females during the following 5 days) the egg strand was fertilized with the sperm of a male from the eastern population.

Fertilized egg-strands were incubated individually in tanks (9 liters, constant supply of tap water at 0.1 liter min^{-1}, temperature 11 °C, raised to 18 °C during the following 4 days, 14 h of light). Three to four days after fertilization, the number of eggs in each tank was reduced from several thousands to approx. 1000 by cutting out a piece of the middle part of every egg-strand. The number of unfertilized eggs was counted for every piece of egg-strand. Unfertilized eggs could easily be identified by their white color and the lack of embryonic development. One to two days after hatching, the number of hatched larvae and of dead embryos, was counted. Finally, the initial number of eggs per tank, the proportion of fertilized eggs and the

proportion of larvae that had hatched from fertilized eggs was calculated.

To determine hybrid fitness, we used a Combined Fitness Measure (CFM) according to Hatfield (1999) multiplying the proportion of fertilized eggs by the proportion of larvae that had hatched from fertilized eggs.

4.3 Results

One to two days after fertilization, embryonic development differed markedly among egg-strands. Some egg-strands, mostly those of the parental populations, were developing well while others, especially those from hybrid crosses showed a high amount of unfertilized eggs. Some days later, deformed embryos were observed in the hybrid crosses, and these embryos eventually died before hatching (Fig. 4.1). We found significant fitness differences between parental and hybrid crosses in the laboratory experiments. The egg fertilization success differed significantly between different crosses (Table 4.1, ANOVA on arcsine square root of proportions, $F_{3,23} = 4.86$, $p = 0.009$). Compared to hybrids $F_1 1$ and the parental populations, hybrids $F_1 2$ had a significantly lower egg fertilization rate (Linear Contrast analysis, $F_{1,23} = 14.29$, $p = 0.001$).

Table 4.1: Mean (±SE) proportion of fertilized eggs and hatched larvae (from fertilized eggs) of different crosses. Eastern population/western population: crosses within subpopulations. F_1-hybrid crosses: crosses between subpopulations, $F_1 1$ mother eastern population/father western population, $F_1 2$, mother western population/father eastern population. N: number of females.

Crosses	N	Prop. fertilized	Prop. hatched	Combined Fitness Measure
Eastern pop	5	0.953 (0.002)	0.651 (0.108)	0.625 (0.104)
Western pop	6	0.985 (0.009)	0.835 (0.049)	0.824 (0.054)
$F_1 1$	7	0.986 (0.005)	0.517 (0.087)	0.510 (0.086)
$F_1 2$	9	0.560 (0.149)	0.507 (0.116)	0.344 (0.098)

The hatching success (proportion of larvae that hatched from fertilized eggs) was lower for both hybrids compared to the parental populations (Table 4.1) although the reduction was not significant (ANOVA on arcsine square root of proportions, $F_{3,23} = 2.14$, $p = 0.122$). The reduction in hatching success could be explained by a high amount of embryos that had died during embryonic development. Especially for hybrid $F_1 1$, a lot of dead embryos remained within the egg shells. The proportion of dead embryos was significantly different for different crosses (ANOVA on arcsine square root of proportions, $F_{3,23} = 4.08$, $p = 0.018$, data not shown). Compared to

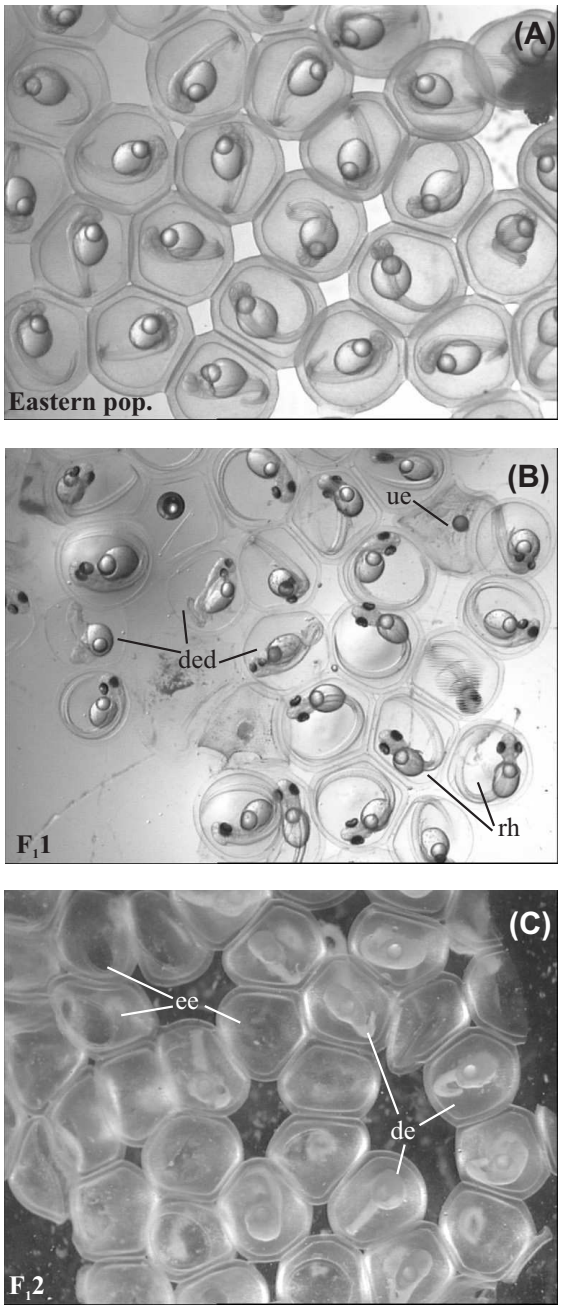

Figure 4.1: Embryonic development of perch from different crosses. (A) Embryos from a parental population (eastern population), four days after fertilization. (B) Egg strand of cross $F_1$1, mother eastern population/father western population, 10 days after fertilization. (rh)= ready to hatch embryos can be seen beneath (ded)= embryos, with disturbed embryonic development and (ue)= unfertilized eggs. (C) Egg strand of cross $F_1$2, mother western population/father eastern population, one day after hatching. (de)= dead whitish embryos of different embryonic stages can be seen beneath (ee)= empty egg shells that remained after hatching.

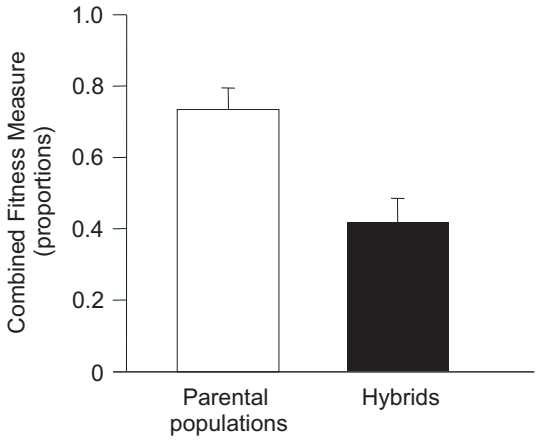

Figure 4.2: Combined Fitness Measure (CFM) for different crosses (parental populations and F_1 hybrid crosses) of perch, belonging to two sympatric populations from Lake Constance.

the western population (6%), hybrids $F_1$1 (42%) and hybrids $F_1$2 (32%) but also the eastern population (29%) had a significantly higher proportion of dead embryos (Linear Contrast analysis, $F_{1,23} = 10.56$, $p = 0.004$).

The CFM differed significantly between different crosses (Table 4,1, Fig. 4.2, ANOVA on arcsine square root of proportions, $F_{3,23} = 5.30$, $p = 0.006$). Compared to the parental populations, hybrid crosses had a significantly lower CFM (Linear Contrast analysis, $F_{1,23} = 10.28$, $p = 0.004$). This was due to both, differences in egg fertilization rate and hatching success.

4.4 Discussion

Our results indicate that some genetic incompatibility has already developed between the two parental populations. Crosses $F_1$1 showed significantly reduced fertilization success (prezygotic isolation) in contrast to crosses $F_1$2. This unidirectional cross-fertilization, where the eggs of one species are receptive to the sperm from the second, but the reverse crosses fail, is common in marine sister species of several sea urchins (Strathmann, 1981; Uehara & Shingaki, 1984; Lessios & Cunningham, 1990) and in *Drosophila* (Coyne & Orr, 1989). Unlike for fertilization, both hybrid crosses showed a reduction in hatching success (postzygotic isolation). Interestingly in contrast to the above mentioned investigations on sea urchins and *Drosophila* that were all done using sister species, we found decreased hybrid fitness already by crossing sympatric populations that may have diverged during no more than the last 10,000 years (Behrmann-Godel et al., 2004). The finding of postzygotic iso-

lation between perch populations is also surprising given the results of Coyne and Orr (1997) who showed that in *Drosophila* sister species prezygotic sexual isolation between species is stronger than postzygotic isolation and that in sympatric species prezygotic isolation is greatly enhanced in comparison to allopatric species.

In sympatric species pairs of sticklebacks, Hatfield and Schluter (1996; 1999) demonstrated that hybrids suffered fitness loss in the wild indicating that primarily ecological and not intrinsic genetic incompatibilities are responsible. Similar experiments with perch hybrids would show, if environment-dependant postzygotic isolation could further reinforce the population splitting of perch in Lake Constance.

However, the underlying mechanisms leading to genetic incompatibilities between species or populations need to be analyzed. The best known examples of underlying mechanisms are the Robertsonian translocation chromosomes in specific races (Rb-races) of the common shrew (*Sorex araneus*) (Hatfield et al., 1992) and the house mouse (*Mus musculus*) in Denmark/Germany (Castiglia & Capanna, 2000). Hybrid zones appear, where a Rb-race comes into contact with a "normal" caryotype or where two Rb-races meet. Hybrids show unusual chromosome configurations that appear at metaphase I because of the lack of homologous chromosomes. The result is a reduced fertility or sterility of hybrids (Said et al., 1993; Hauffe & Searle, 1998). Moreover, by germ cell death in both sexes (Redi et al., 1985; Garagna et al., 1989), heterozygote hybrids are expected to have a reduced lifetime reproductive success. Hybrid fitness could also be influenced by epistatic effects and/or linkage disequilibrium (Maynard Smith, 2001). Hereby, high fitness would require that several genes, at different loci, be all present. If one of these genes would be altered by hybridization, fitness of hybrids would decrease.

In contrast to hybrid crosses $F_1$1, we conducted fertilization for six of nine hybrid crosses $F_1$2 under laboratory conditions. We kept not yet ripe females from the western population in a tank until their egg-strands could be used for artificial fertilization. We cannot rule out that this method resulted in less fit hybrid offspring of these specific crosses. However, a statistic analysis excluding these six crosses did not alter our outcome of reduced hybrid fitness compared to the parental populations (data not shown).

Our results show that genetic divergence between sympatric perch populations in Lake Constance may be reinforced by unconditional isolation based on reduced hybrid fitness. Population structuring has also been found for perch (Bodaly et al., 1989) and yellow perch (*Perca flavescens*) (Miller, 2003) in Lake Windermere and Lake Michigan respectively. Maybe population divergence of perch and yellow perch in these two lakes could also be reinforced by reduced hybrid fitness.

Chapter 5

Preliminary investigation on morphological differences between sympatric populations of perch (*Perca fluviatilis* L.)

5.1 Introduction

Variance in morphological traits within a species can be found in a variety of vertebrates and seem to be correlated with differences in resource use. These "trophic polymorphisms" are very common especially in fish (Skulason, 1995; Smith, 1996; Riffel, 1998). Recent investigations have shown that these polymorphisms often occur among sympatric species pairs (Schliewen et al., 2001; Robinson & Parsons, 2002). Different morphs can reflect distinct ecotypes that are adapted to different ecological niches within the same habitat. This has been shown for sympatric forms of many freshwater fish species (including Eurasian perch) especially in northern postglacial lakes (for a review see (Robinson & Parsons, 2002). The common pattern of divergence is the evolution of benthic (benthivorous) and limnetic (planktivorous) morphs like e.g. in coregonids (Bernatchez et al., 1999), Icelandic arctic charr (*Salvelinus alpinus*)(Gislason et al., 1999), threespine sticklebacks (*Gasterosteus aculeatus*) (Schluter, 1996) and perch (*Perca fluviatilis*)(Hjelm et al., 2001). In Icelandic Arctic charr, an additional piscivorous morph was found in one of the lakes. In *Percichthys trucha*, two benthic morphs, a littoral and a deep benthic morph were found in a series of lakes in the Andes (Ruzzante et al., 1998). In sticklebacks, hybrids of the two forms seem to be less well adapted to the ecological niches of their parent populations and are selected against (Rice & Hostert, 1993).

These findings have changed the view of how speciation can occur. Despite the widely accepted theory of allopatric speciation where populations diverge during geographical isolation, models have been developed to explain how divergence can occur in populations living in sympatry (Dieckmann & Doebeli, 1999; Kondrashov

& Kondrashov, 1999). In support of these theoretical models, empirical evidence for sympatric speciation is accumulating rapidly (for a review see Via, 2001).

This study focuses on Eurasian perch (*Perca fluviatilis* L.) a very common freshwater fish species with a wide distribution that occupies a variety of different habitats in the northern temperate hemisphere (Thorpe, 1977). Hjelm et al. (2000, 2001) could show that perch morphology can be very variable and is related to the resource base used. The authors studied perch morphology in lakes with different trophic states and as a consequence differences in the frequency of planktivorous competitors. Their study suggests a morphological trade-off between the use of the benthivorous and the planktivorous niche. Additionally predation risk can have an influence on the body morphology of young-of-the-year (YOY) perch (Magnhagen & Heibo, 2004).

In Lake Constance, two genetically different perch populations ($G_{ST} = 0.07$) coexist. One population inhabits the eastern (eastern population) the other the western part of the lake (western population). Since no geographical barrier separates the populations, they live in sympatry (Gerlach et al., 2001). The two habitats for the sympatric perch populations in Lake Constance are the two lake basins Upper and Lower Lake Constance. They are very different in terms of basin morphology and trophic state. Upper Lake Constance is deep, oligotrophic and warm monomictic, while Lower Lake Constance is much shallower, mesotrophic and dimictic. Perch in Lake Constance might have divided into distinct ecotypes that have adapted to different ecological niches within the tow basins of the lake.

The aim of our study was to investigate, whether the genetically distinct subpopulations also show morphological differences, these could reflect different ecotypes that have formed as a consequence of ecological character displacement (Schluter & McPhail, 1992).

5.2 Materials and methods

We caught 109 adult perch(55 of the western, and 54 of the eastern population) of approx. 20 cm body length in the littoral zone of Lake Constance using gill nets. Fish were transported to the laboratory on ice, positioned on plotting paper and photographed with a digital camera. Using a digital frame grabber and self-developed software, morphometric parameters could be measured to the nearest 0.2 mm (except for the intraorbital width (D 17-18), the height of the third ray in the first dorsal fin (D 13-14) and the mouth width (D 15-16), which were conventionally measured to the nearest 0.5 mm using a caliper). Fifteen morphometric and five meristic traits were evaluated. Morphometric traits are shown in Fig. 5.1. All

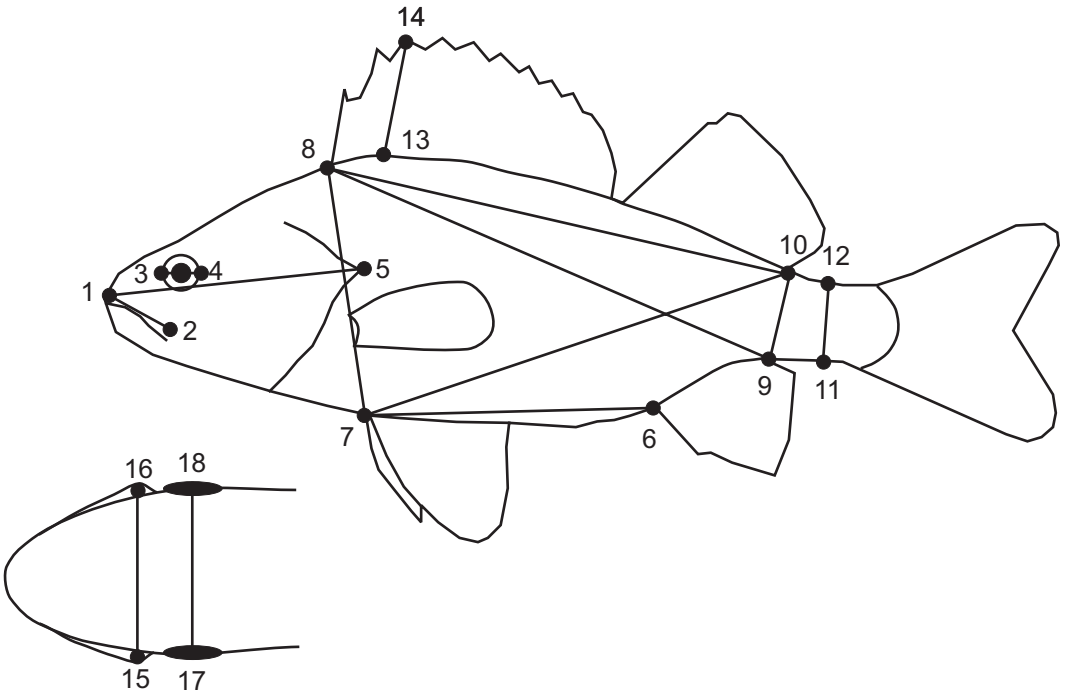

Figure 5.1: Morphometric landmarks used in the analysis of variation between two subpopulations of perch in Lake Constance. Most common variables in detail: D 1-2 upper jaw length; D 3-4 orbital diameter; D 1-5 head length; D 7-8 body height anterior to the dorsal fin; D 9-10 body height posterior to the dorsal fin; D 11-12 height of the caudal peduncle; D 13-14 height of the first dorsal fin (measured at the third fin ray); D 15-16 mouth width; D 17-18 intraorbital width.

morphometric parameters were standardized to the total length according to the formula of Riffel & Schreiber (1998):

$$\mathrm{M}_S = \mathrm{M}_0(\mathrm{L}_S/\mathrm{L}_0)^b,$$

where M_S denotes the standardized measurement, M_0 the observed measurement, L_S the arithmetic mean for all specimen examined, L_0 the standard length of the specimen and the regression coefficient b was estimated as the slope of the regression of log M_0 on log L_0. Meristic parameters were: the amount of vertical stripes on the body, the gill raker number and the numbers of rays in the first and second dorsal and the anterior fins.

For statistical analysis, we did not distinguish between sexes. Morphometric and meristic data were analyzed separately. We first tested for normality of the standardized morphometric characters (Kolmogoroff-Smirnof test). If normality could be verified, pairwise means of the two subpopulations were tested for significant de-

viations. Nonparametric tests were used if variances deviated from normality. Since none of the meristic characters was normally distributed, these were exclusively examined by nonparametric U-test.

5.3 Results

Morphometric characters

The results of the statistical comparison of the morphometric characters between the two subpopulations are shown in Table 5.1. We found significant differences in four of the 13 characters. The upper jaw (D 1-2) was longer in the eastern population. The following characters were all larger for the western population: length of the dorsal fin (D 8-10), distance between the anterior end of the dorsal fin base and the posterior end of the anal fin base (D 8-9), and height of the dorsal fin, measured at the third fin ray (D 13-14).

Table 5.1: Morphometric traits of perch belonging to two subpopulations (western- and eastern population) of Lake Constance see also Fig. 5.1.

	Western population		Eastern population			
Trait	Mean [mm]	SD	Mean [mm]	SD	Statistics	p-value
D 1-2	16.01	1.71	16.70	1.73	t-test	0.036*
D 3-4	9.81	0.73	10.10	0.88	t-test	0.063
D 1-5	47.24	2.59	48.24	3.15	U-test	0.072
D 7-7	46.41	1.83	46.45	1.89	t-test	0.910
D 11-12	14.18	0.67	14.12	0.61	t-test	0.636
D 9-10	21.73	1.08	21.42	1.07	t-test	0.130
D 8-10	98.15	2.92	96.94	2.92	t-test	0.033*
D 8-9	99.31	2.46	97.83	2.41	t-test	0.002**
D 7-10	95.75	1.90	95.39	2.09	t-test	0.340
D 6-7	67.14	2.36	67.19	3.04	Welch test	0.931
D 13-14	24.94	1.60	23.50	1.71	t-test	< 0.001***
D 17-18	18.39	1.26	18.43	1.70	U-test	0.898
D 15-16	16.14	1.77	16.34	1.44	U-test	0.511

Meristic characters

Comparisons of subpopulations by means of nonparametric tests yielded significant differences in four of the five meristic variables (Table 5.2). The amount of stripes (Str) and the number of rays in all three types of fins measured (D1FR, D2FR, AFR), were significantly higher in the eastern as compared with the western population.

Table 5.2: Meristic traits of perch belonging to two subpopulations (western and eastern population) of Lake Constance. STR, number of stripes; GR, number of gill rakers; D1FR, number of fin rays in the first dorsal fin; D2FR, number of fin rays in the second dorsal fin; AFR, number of fin rays in the anterior fin.

	Western population			Eastern population			
Trait	Mean	SD	Median	Mean	SD	Median	Significance
STR	9.12	1.27	9	8.76	1.05	9	*
GR	23.33	1.29	23.5	23.15	1.42	23	NS
D1FR	14.56	0.74	15	14.16	0.66	14	***
D2FR	15.19	0.75	15	15.04	0.67	15	*
AFR	10.54	0.54	11	10.35	0.58	10	***

U-test: *P <0.05; ***P<0.001; NS = non significant

5.4 Discussion

The statistical analysis demonstrated morphological variation between the two sympatric subpopulations of perch in Lake Constance. However, only four out of 13 morphometric characters showed significant differences and the absolute differences were very small (1-2 mm, Table 5.1). In contrast, only one meristic trait, gill raker number did not significantly differ between the two populations (Table 5.2). The morphological comparison between sympatric ecotypes of whitefish and sticklebacks revealed much more pronounced results (Lu & Bernatchez, 1999; McPhail, 1993). Additionally, the morphological differences between perch subpopulations in Lake Constance did not concern traits that have been shown to be the aim of trade-offs that are correlated to resource use (Hjelm et al., 2000, 2001). Hjelm et al. (2001), have shown that a deeper body is characteristic of benthivorous feeding and for feeding in the littoral habitat, whereas piscivorous perch have a more slender body shape. A slender body form was also reported to be related to planktivorous feeding in perch that inhabit the pelagic zone (Eklöv & Persson, 1995; Hjelm et al., 2000). All perch used in this study (and also in the study on genetic differentiation of perch by Gerlach et al., 2001) were caught in the littoral zone of the lake. Referring to the differences in lake basin morphology and trophic state, we had expected that perch from the eastern population would be more adapted to the piscivorous niche and perch from the western population to the benthivorous niche. However, we could not find any discrepancy between traits correlated to body height (D 7-8, D 9-10) or height of the caudal peduncle (D 11-12; Table 5.1). Gape width is an important constraint on prey use and a large gape is adaptive to piscivores (Wainwright & Richard, 1995; Christensen, 1996). For perch, a positive relationship of gape width

to the amount of planktivorous prey was found (Hjelm et al., 2000). This indicated that piscivorous perch have larger gape widths. In our study, no difference was found for mouth widths (D 15-16; Table 5.1) between the two subpopulations, but the eastern population had a significantly longer upper jaw (D 1-2; Table 5.1), which could be an indicator for a slightly larger gape. Hjelm and colleagues found an indication for an increased gill raker number in young-of-the-year perch that were adapted to the planktivorous niche (Hjelm et al., 2000, 2001). In our study no differences were found for gill raker number (GR; Table 5.2) or gill raker length (data not shown). A comparison of the stomach contents showed that adult perch from both subpopulations were almost exclusively piscivorous, feeding mainly on young conspecifics (data not shown). It is common opinion that perch have to pass through a zooplankton as well as through an invertebrate feeding stage to reach piscivory (ontogenetic niche shift). This means an ontogenetic trade-off is present that is most important between the benthivorous and the piscivorous stage, because of the distinct differences in body morphology of perch that are best adapted to these two niches (Hjelm et al., 2000). Adult perch of Lake Constance belonging to both subpopulations are mainly piscivorous, the benthic deeper-bodied perch may be sub-optimal and therefore recruitment to the piscivorous niche could be reduced.

However, the most prominent differences between the two subpopulations were found in the sizes of the dorsal and anal fins. We found more fin rays in all three types of fins of the western population, and this population also expressed a higher dorsal fins than the eastern population. The size of fins has been shown to contribute to the overall body shape of fishes (Robinson & Parsons, 2002) and could thus also account for a deeper body of perch from the western population. Magnhagen & Heibo (2004) showed that in lakes where the predation risk by pike and cannibalistic perch was high, YOY perch had higher body depths and dorsal fin ray lengths compared to perch in lakes with low predation risk. Because of lower water depth and an extended area of the littoral zone of Lower Lake Constance, the number of pike could be increased compared to Upper Lake Constance. A higher dorsal fin of perch from Lower Lake Constance (western population) could be a adaptation to higher predation risk by pike. An extended morphological study using digital landmarks and investigations on predator density in the two lake basins might test this hypothesis.

Morphological variance between the two perch subpopulations in Lake Constance was not very pronounced although it seems to be associated to genetic differences found in the previous study of Gerlach et al. (2001). Genetic variation is a prerequisite for selection to have an evolutionary consequence in any trait being considered.

Empirical evidence of genetic variation for morphological plasticity in fishes could be shown for different freshwater fish species. Day et al. (1994) reared species pairs of threespine sticklebacks on different diets and could show that (i) morphologic plasticity is adaptive, (ii) the species with the more variable diet in the wild provided greater morphological plasticity, which is strong evidence that diet variability is important in the evolution of plastic trophic morphology, (iii) that genetic variation for morphological plasticity existed in contemporary populations indicating that plastic morphology has evolutionary potential. Heritable genetic variation could also be shown for pumpkinseed sunfish (Robinson & D.S., 1996) and chum salmon (Beacham, 1990).

From our previous investigation we conclude that the slight but significant morphological differences between the two subpopulations in Lake Constance do so far not prove the hypothesis of ecological speciation between the two subpopulations. However, they could still reflect the underlying genetic differences (Skúlason et al., 1999) and be the result of genetic drift or of an accumulation of genetic differences due to restricted gene flow of reproductively isolated subpopulations.

Chapter 6

Discussion and suggestions for further research

The major outcomes of this thesis are the following.

- Perch subpopulation structure in Lake Constance is most likely the result of sympatric population splitting after colonization by a single source population during the last deglaciation that started approx. 15,000 years ago.
- The kin structure found in perch aggregations could be due to an active choice of kin as shoal mates and be based on olfactory recognition. The same sensory mechanism could account for population recognition and play a major role in enhancing subpopulation structure.
- F_1hybrids suffer fitness losses in the form of reduced fertilization and hatching success of cross matings between the two subpopulations. A reduction in hybrid fitness could reinforce population divergence between perch subpopulations.
- A preliminary investigation on adult perch morphology revealed slight but significant differences between the eastern and western subpopulations, indicating an accumulation of genetic incompatibilities between subpopulations.

In the previous chapters, the results of my experiments were discussed with respect to the specific topic and related studies. In the following chapter, I want to give some general and maybe sometimes slightly speculative conclusions in the context of perch population subdivision in Lake Constance. I will discuss my findings in relation to commonly known mechanisms for sympatric divergence like ecological speciation and sexual selection. I want to show that with the research on evolutionary mechanisms that drive population divergence in perch I discovered empirical evidence for a hitherto only theoretically discussed mechanism that might drive sympatric speciation.

The coexistence of two different populations within a lake might result either from secondary contact of two different source populations that have diverged in allopatry, or from sympatric divergence within the lake. The mitochondrial DNA analysis (mtDNA D-loop sequencing) in combination with the geological data strongly suggest a sympatric origin of perch subpopulations. The analysis of the variability of mtDNA showed no significant difference in haplotype frequencies between the eastern and western subpopulations. After the withdrawal of the Pleistocene glacier and the formation of Lake Constance approx. 15,000 – 10,000 years ago, both parts of the lake have been permanently interconnected, which would have allowed a continuous genetic exchange. I thus conclude that Lake Constance perch originated from a single source (the Danube population) and started diverging sympatrically in the lake. The question arises, which mechanisms have been developed to maintain and/or drive population subdivision.

For sympatric populations it can be expected that the subpopulations form clusters of different ecotypes that are adapted to different ecological niches within the habitat. This could be shown for sympatric forms of coregonids (Bernatchez et al., 1999) and sticklebacks (Schluter, 1996). In these species, significant morphological differences reflect the existence of sympatric benthic and limnetic forms that are reproductively isolated. Hybrids, if they appear, are less well adapted to the ecological niches of their parent populations and are selected against (Rice & Hostert, 1993).

The two main habitats for the sympatric perch populations in Lake Constance are the two lake basins Upper and Lower Lake Constance (See Fig. 2.2 in chapter 2). They are different in terms of lake basin morphology and trophic state. Upper Lake Constance is deep, oligotrophic and warm monomictic, while Lower Lake Constance is much shallower, mesotrophic and dimictic. The genetically distinct subpopulations could thus reflect different ecotypes that have formed on the basis of character displacement (Schluter & McPhail, 1992). Discrepancies in morphological traits between the distinct ecotypes often reflect adaptations to different food resources, competitors or predators (Robinson, 2002).

Hjelm et al. (2000, 2001) could show that perch morphology can be very variable and is related to the resource base used. The authors studied perch morphology in lakes with different trophic states and as a consequence differing amounts of planktivorous competitors. Their study suggests a trade-off between the benthic and the piscivore niche whereby a deeper body can be found for perch in the benthic and a more fusiform body in the piscivorous niche. Additionally, a larger gape width was found for piscivorous perch.

Our preliminary study on differences in morphological characters shows significant differences between the two subpopulations, but is so far not very convincing to prove ecological speciation between perch subpopulations in Lake Constance.

However, a well known consequence of the striking differences in lake basin morphologies directly leads to differences in the spawning time of the two subpopulations. Perch of the western population spawn about two weeks earlier then perch of the eastern population, due to a faster warming up of the water in the shallower lower lake (K. Egloff personal, communication and own observations). Asynchrony in spawning times is considered an effective premating isolation mechanism for sympatric sibling species (for a review see Knowlton, 1993; Knowlton et al., 1997; Palumbi, 1994), but the causes of this asynchrony are often not known. In perch, the asynchrony appears to be directly related to spawning temperature leading to spatial segregation of perch groups hatching at different times in different locations.

We conclude that although we do not find distinct ecotypes of perch that differ by morphological traits, we could still find ecological separation that is based on differences in spawning time due to different temperature regimes.

In their theoretical models for sympatric speciation Diekmann & Doebeli (1999) and Kondrashov & Kondrashov (1999) have shown that association between traits that are under ecological selection and neutral traits that are used for mate choice is fundamental, which may then lead to assortative mating. Only the association of these traits can suppress the mixture of gene pools and drives population divergence. Thus it is commonly accepted that ecological speciation in combination with sexual selection can be the basis for sympatric speciation (see cichlid diversity below). For Eurasian perch, sexual dimorphism has never been reported and neither males nor females show any visual secondary sexual traits during spawning time. This left open the question, which putative male traits females could use for assortative mating. A possibility is that they use non-visual traits like e.g. specific odors due to MHC polymorphism. This has shown to be a mechanism for sexual selection in sticklebacks (Reusch et al., 2001). Differences in male mating behavior might be another trait used in female mate choice as has also been found in sticklebacks belonging to two sympatric forms (Hatfield & Schluter, 1996). Further studies on perch mate choice and mating behavior would be important to test for differences between the two subpopulations.

However, in the following I will introduce a new mechanism that, besides sexual selection, can result in assortative mating and can drive sympatric population splitting in perch.

According to my results sympatric divergence of perch in Lake Constance is on a very early stage, since 10,000 years is not very much time on an evolutionary time scale. However, the cichlids of the East African lakes evolved hundreds of different species during comparable time spans and recent studies suggest that both, adaptation to different niches (Schliewen et al., 1994) and sexual selection (Seehausen & van Alphen, 1999) have played significant roles in their speciation (reviewed in Meyer, 1993). Probably during the very early state of perch speciation, other evolutionary mechanisms with much slower divergence rates play an important role in contrast to the ones that have been found for cichlids.

Two populations undergoing speciation must be separated by reproductive isolation. Reproductive isolation happens if individuals of one subpopulation avoid to mate with individuals of the other subpopulation (leading to assortative mating). Our finding that juvenile perch recognize and prefer kin by waterborne olfactory cues and additionally recognize and prefer members of the own subpopulation could present a new mechanism, which can lead to the separation of gene pools and to the formation of new species in the future. Kin recognition and population recognition may have the same chemosensory basis, i.e. the recognition and preference of family specific odors. For juvenile fish, kin recognition could be advantageous if co-operative behavior e.g. during predator inspection increases an individual's inclusive fitness (Hamilton, 1964). Nevertheless, for adult fish mating with close relatives can lead to inbreeding and is thus disadvantageous due to the increase of homozygosity and the possible expression of recessive deleterious mutations in offspring (Charlesworth & Charlesworth, 1987). Kin recognition can therefore be advantageous in different directions. Individuals may profit from the benefits of co-operation with relatives and on the other hand can minimize inbreeding depression by avoiding to mate with full- and half-sibs and rather mate with first or second cousins (Laland, 1994). F_{IS} values of local perch populations in Lake Constance indeed showed significant deviations from Hardy-Weinberg equilibrium but had negative values (average $F_{IS} = -0.117$, for details see Gerlach et al., 2001). This indicates an increase in the frequency of heterozygotes and not an increase in the frequency of homozygotes, as would be expected, if inbreeding would occur. Thus, the ability of juvenile perch to discriminate between the own and the foreign population could be an early manifestation of a behavior that will be exploited later in live during mating. A similar behavior has also been suggested for salmon fry (Courtenay et al., 1997). Olfactory discrimination would then lead to assortative mating and avoid inbreeding at the same time. This would be a perfect mechanism to maximize individual perch reproduction success and would as a byproduct reinforce reproductive isolation of the two subpopulations.

Thus the experiments on kin and population recognition present empirical evidence for a hitherto only theoretically discussed mechanism that leads to reproductive isolation. A model of "socially mediated speciation" by Hochberg et al. (2003) proposed that social behaviors i.e. altruistic and selfish acts can cause the congealing of phenotypically similar individuals into different, spatially distinct tribes. In our study we suggest such a behavioral mechanism: kin and group recognition leading to assortative mating. The time span for Lake Constance perch living in sympatry, approx. 10,000 years i.e. at least 3300 generations, is well within the time span of approx. 1000 generations that has been found by Hochberg and colleagues to be sufficient for mating preference to reach fixation and to result in reproductive isolation. Whereas the idea of "socially mediated speciation" has been discussed in evolutionary biology (West-Eberhard, 1983), I believe that ours is the first empirical study showing that social preferences might drive population divergence.

Mate choice experiments with females and males that have the choice between mating partners, which belong to the same and to the foreign population could show, whether assortative mating indeed occurs in perch.

Our laboratory experiments on hybrid fitness showed that some genetic incompatibilities have already accumulated between the parent populations. Therefore, genetic divergence between the two perch subpopulations in Lake Constance could be reinforced by reduced hybrid fitness. The reduction in hybrid fitness in combination with kin recognition and the formation of kin aggregations could be another indicator for a socially mediated speciation process in perch. In the socially mediated speciation model of Hochberg et al. (2003), hybrid fitness decreases following the invasion of a new trait. In their model, lowered hybrid fitness drives social selection and the outcome is assortative mating leading to reproductive isolation between populations.

Since perch spawn only once a year and females lay all their eggs in only one spawning event, it seems obviously urgent to find the right spawning partner to avoid fitness losses of the offspring due to hybridization or inbreeding.

Conclusion

The results of my studies represent the first empirical evidence that socially mediated divergence in combination with ecological factors can explain population splitting. Perch in Lake Constance spawn in the lake's littoral zones in early spring. The local temperature regimes cause the western population to spawn about two weeks earlier and form shoals of larvae well before eastern shoals emerge. Local groups hatch approx. synchronously resulting in coherent shoals of larvae that are largely composed of closely related animals. This relatedness persists in juvenile and adult

shoals as was shown in a previous genetic study (Gerlach et al., 2001). Group preference is based on olfactory preference for related individuals and if it lasted during spawning time, this behavior could lead to assortative mating. Assortative mating in turn, maintains and enhances the separation between the eastern and western populations. The separation is further enhanced by selection against hybrids due to genetic incompatibilities between the two subpopulations. This interpretation suggests that perch speciation in Lake Constance is currently underway. The combination of 1) ecological separation based on differences in spawning time/location and 2) kin/population recognition mechanisms might therefore be an effective basis for sympatric speciation.

Chapter 7

Summary

The central issue of this thesis is the understanding of evolutionary mechanisms, that maintain and drive the divergence of populations and can lead to sympatric speciation. A former study showed that two populations of perch (*Perca fluviatilis* L.) co-exist in Lake Constance. For the first time I provide empirical evidence, that socially mediated divergence (kin- and population preference) in combination with ecological factors (difference in spawning times) could explain the origin and persistence of the perch subpopulations. Divergence between perch populations could be reinforced by reduced hybrid fitness.

My results support that both populations originated from a single source population because analysis of the variability of mitochondrial DNA (mtDNA D-loop sequencing) showed no difference in haplotype frequencies between the eastern and western subpopulations of perch in Lake Constance. Moreover, perch originated most likely from the Danube River because Danube and Lake Constance perch share the same distinct haplotype, whereas perch from the Rhine (the second possible refuge) lack this special haplotype. This is consistent with the geological history of Lake Constance with a known connection between the lake and the Danube during lake formation in the Pleistocene (approx. 15,000 years ago). Subsequent upstream colonization from the Rhine is unlikely because there has always been a impassable waterfall.

A microsatellite analysis on the genetic structure of larval perch in the pelagic zone of Lake Constance showed that perch form shoals of closely related conspecifics. To test if this behavior is due to a preference for members of their own subpopulation, including kin, I investigated active choice for olfactory cues in laboratory reared perch. Juvenile perch were tested in a two-channel flume for preference of odors from different conspecifics of known relatedness. Three different choice experiments were conducted: holding water from kin (three different sibling groups were tested: full-sibs, maternal half sibs or paternal half sibs) was always tested vs. holding water

of non-kin belonging to the same subpopulation. Perch significantly preferred kin over non kin, there was no difference between the different kinship tests.

In a second experiment population recognition was tested. Juvenile perch significantly preferred unrelated members of their own versus members of the foreign population. From these experiments I conclude that kin and population coherence can be an active choice based on olfactory preference, which could easily lead to assortative mating.

An important constraint on disruptive population divergence is selection against hybrids. Therefore I measured the fertilization and hatching success of F_1 hybrids of the two populations compared to those within each parental population. Two different F_1 hybrids (mother eastern population/father western population and vice versa) were produced by artificial fertilization. Compared to the eastern and western populations, fitness was significantly lower for both hybrids. These laboratory measures of hybrid fitness suggest that some genetic incompatibility has already accumulated in the parental populations. Therefore, genetic divergence between the two perch populations in Lake Constance seems to be underway and is reinforced by reduced hybrid fitness.

Differences in lake basin morphology could lead to ecological separation of the two subpopulations based on asynchrony in spawning time/location. In a preliminary morphological analysis slight but significant differences could be found between the two subpopulations. Differences in morphological traits did not affect characters, that are commonly driven by ecological speciation but reflect an accumulation of neutral genetic differences between reproductively isolated subpopulations.

To conclude, I provide empirical evidence for a socially mediated divergence, that may drive sympatric speciation of perch: asynchrony in spawning time and location cause ecological separation of perch into subpopulations. The resulting disruptive population divergence is enhanced by olfactory preference for kin and conspecifics of the same population, and is reinforced by selection against hybrids.

Zusammenfassung

Zentrales Thema dieser Dissertation ist das Verständnis evolutionärer Mechanismen, welche die Aufspaltung von Populationen aufrecht erhalten und vorantreiben und zu sympatrischer Artbildung führen können. Eine vorangegangene Studie zeigte, dass im Bodensee zwei Populationen von Barschen (*Perca fluviatilis* L.) koexistieren. Erstmals wird durch meine Untersuchungen ein empirischer Nachweis erbracht, dass sozial-vermittelte Mechanismen (Bevorzugung von Verwandten und Bevorzugung der eigenen Population) in Kombination mit ökologischen Faktoren (Unterschiede in den Laichzeiten) die beobachtete Populationsaufspaltung der Barsche im Bodensee erklären könnten. Diese Aufspaltung könnte durch eine Reduktion in der Fitness der Hybride zwischen den beiden Populationen eine Verstärkung erfahren.

Meine Untersuchungen legen nahe, dass beide Populationen der gleichen Ursprungspopulation entstammen, denn Untersuchungen der Variabilität mitochondrieller DNS (mtDNS D-loop Sequenzierung) erbrachten keine Unterschiede in den Haplotypfrequenzen zwischen der östlichen und der westlichen Barschsubpopulation. Die Barsche im Bodensee entstammen sehr wahrscheinlich der Donau, denn bei Barschen aus dem Bodensee und der Donau wurde der gleiche mtDNS Haplotyp gefunden, während dieser spezielle Haplotyp bei Barschen aus dem Rhein (des zweiten möglichen Rückzuggebietes) fehlt. Diese Befunde stimmen mit der geologischen Geschichte des Bodensees überein, in der eine Verbindung zwischen See und Donau während der Entstehung des Sees, im Pleistozän (vor ca. 15,000 Jahren) bekannt ist. Eine nachfolgende, stromaufwärts führende Besiedelung vom Rhein her ist unwahrscheinlich, da ein unpassierbarer Wasserfall eine solche schon immer unmöglich gemacht hat.

Eine Mikrosatelliten Untersuchung der genetischen Struktur von Barschlarven aus dem Pelagial des Bodensees zeigte, dass Barsche Schulen mit eng verwandten Artgenossen bilden. Um zu testen, ob dieses Verhalten aus einer Präferenz für Artgenossen der gleichen Population, und von Verwandten resultiert, untersuchte ich eine aktive Wahl von Duftstoffen. Hierfür wurden juvenile Barsche in einem Strömungskanal auf ihre Präferenz für Duftstoffe von unterschiedlich verwandten

Artgenossen untersucht. Es wurden drei verschiedene Wahlversuche durchgeführt: Hälterungswasser von Verwandten (drei verschiedene Verwandtschaftsgruppen wurden getestet: Vollgeschwister, maternale Halbgeschwister und paternale Halbgeschwister) wurde jeweils gegenüber Hälterungswasser von nicht Verwandten der gleichen Subpopulation getestet. Die Testfische zeigten eine signifikante Präferenz von Verwandten gegenüber nicht Verwandten, es gab keine Unterschiede zwischen den verschiedenen Verwandtschaftstests. In einem zweiten Experiment wurde die Populationserkennung untersucht. Juvenile Barsche bevorzugten signifikant unverwandte Angehörige der eigenen Subpopulation gegenüber Angehörigen der fremden Subpopulation. Aus diesen Experimenten schliesse ich, dass der Zusammenhalt mit Verwandten und Angehörigen der eigenen Subpopulation eine aktive Wahl darstellen kann, welche auf einer olfaktorischen Präferenz beruht. Dieses Verhalten könnte zu einer Verpaarung mit phänotypisch ähnlichen Artgenossen führen.

Eine wichtige Voraussetzung für sich aufspaltende Populationen ist die Selektion gegen Hybride. Aus diesem Grund untersuchte ich den Befruchtungs- und Schlupferfolg von F_1 Hybriden der beiden Subpopulationen im Vergleich zu den Elternpopulationen. Durch künstliche Befruchtung wurden zwei verschiedene F_1 Hybride (Mutter östliche, Vater westliche Population und andersherum) erzeugt. Im Vergleich zu der östlichen und westlichen Elternpopulation war die Fitness beider Hybride signifikant erniedrigt. Diese Laboruntersuchungen zur Fitness von Hybriden legen den Schluss nahe, dass sich bereits einige genetische Inkompatibilitäten zwischen den Elternpopulationen angesammelt haben. Eine genetische Aufspaltung zwischen den beiden Barschpopulationen im Bodensee scheint begonnen zu haben und diese wird durch selektive Prozesse gegen Hybride verstärkt.

Unterschiede in der Morphologie der beiden Seebecken könnte zu ökologischer Aufspaltung der beiden Subpopulationen führen, die auf asynchronen Laichzeiten und Laichorten beruht. In einer ersten morphologischen Untersuchung wurden geringfügige aber signifikante Unterschiede zwischen den beiden Subpopulationen gefunden. Die morphologischen Unterschiede betrafen keine Merkmale, die allgemein durch ökologische Speziation betroffen sind aber sie stellen eventuell eine Akkumulation neutraler genetischer Unterschiede dar, die sich zwischen reproduktiv isolierten Populationen angehäuft haben.

Als Fazit aus meinen Untersuchungen erbringe ich einen empirischen Hinweis für eine Populationsaufspaltung, die durch soziale Mechanismen vorangetrieben wird: Unterschiede in Laichzeit und Laichort bedingen eine ökologische Aufspaltung der Barsche in Subpopulationen. Diese Aufspaltung wird durch olfaktorische Präferenz für Verwandte und Angehörige der eigenen Subpopulation vorangetrieben und erfährt

eine Verstärkung, durch die Selektion gegen Hybride.

Chapter 8

References

Arnold, K. E. 2000. Kin recognition in rainbowfish (*Melanotaenia eachamensis*): sex, sibs and shoaling. *Behavioral Ecology and Sociobiology*, **48,** 385-391.

Atema, J., Kingsford, M. & Gerlach, G. 2002. Larval reef fish could use odour for detection, retention and orientation to reefs. *Marine Ecology Progress Series*, **241,** 151-160.

Avise, J. C. 1992. Molecular population structure and the biogeographic history of a regional fauna a case history with lessons for conservation biology. *Oikos*, **63,** 62-76.

Avise, J. C. & Shapiro, D. Y. 1986. Evaluating kinship of newly settled juveniles within social groups of the coral reef fish *Anthias squamipinnis*. *Evolution*, **40,** 1051-1059.

Ayala, F. J. & Fitch, W. M. 1997. Genetics and the origin of species: An introduction. *National Academy of Sciences of the United States of America*, **94,** 7691-7697.

Balling, T. E. & Pfeiffer, W. 1997. Location-dependent infection of fish parasites in Lake Constance. *Journal of Fish Biology*, **51,** 1025-1032.

Barnett, C. 1986. Rearing conditions affect chemosensory preferences in young cichlid fish. *Ethology*, **72,** 227-235.

Barton, N. H. 2001. The role of hybridization in evolution. *Molecular Ecology*, **10,** 551-568.

Barton, N. H. & Charlesworth, B. 1984. Genetic revolutions, founder effects and speciation. *Annual Revue of Ecology and Systematics*, **15,** 133-164.

Beacham, T. D. 1990. A genetic analysis of meristic and morphometric variation in chum salmon (*Oncorhynchus keta*) at three different temperatures. *Canadian Journal of Zoology*, **68,** 225-229.

Behrmann-Godel, J., Gerlach, G. & Eckmann, R. 2004. Postglacial colo-

nization shows evidence for sympatric population splitting of Eurasian perch (*Perca fluviatilis* L.) in Lake Constance. *Molecular Ecology*, **13**, 491-497.

Belkhir, K., Borsa, P., Goudet, J., Chikhi, L. & Bonhomme, F. 1997. Genetix v. 3.0, logiciel sous Windows TM pour la génétique des populations. Montpellier: Laboratoire Génome et Populations, CNRS UPR 9060, Université Montpellier 2.

Belkhir, K., Castric, V. & Bonhomme, F. 2002. IDENTIX, a software to test for relatedness in a population using permutation methods. *Molecular Ecology Notes*, **2,** 611-614.

Bernatchez, L. 2001. The evolutionary history of brown trout (*Salmo trutta* L.) inferred from phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA Variation. *Evolution*, **55,** 351-379.

Bernatchez, L. & Wilson, C. C. 1998. Comparative phylogeography of Nearctic and Palearctic fishes. *Molecular Ecology*, **7,** 431-452.

Bernatchez, L., Chouinard, A. & Lu, G. 1999. Integrating molecular genetics and ecology in studies of adaptive radiation: Whitefish, *Coregonus sp.*, as a case study. *Biological Journal of the Linnean Society*, **68,** 173-194.

Bodaly, R. A., Ward, R. D. & Mills, C. A. 1989. A genetic stock study of perch, *Perca fluviatilis* L., in Windermere. *Journal of Fish Biology*, **34,** 965-967.

Borer, S. O., Miller, L. M. & Kapuscinski, A. R. 1999. Microsatellites in walleye *Stizostedion vitreum. Molecular Ecology*, **8,** 336-338.

Brown, J. A. & Colgan, P. W. 1986. Individual and species recognition in centrarchlid fishes: evidence and hypotheses. *Behavioral Ecology and Sociobiology*, **19,** 373-379.

Brown, G. E. & Brown, J. A. 1992. Do rainbow trout and Atlantic salmon discriminate kin? *Canadian Journal of Zoology*, **70,** 1636-1640.

Brown, G., E., Brown, J., A. & Crosbie, A., M. 1993. Phenotype matching in juvenile rainbow trout. *Animal Behaviour*, **46,** 1223-1225.

Brown, G. E. & Brown, J. A. 1993. Do kin always make better neighbors?: the effects of territory quality. *Behavioral Ecology and Sociobiology*, **33,** 225-231.

Brown, G. E. & Brown, J. A. 1996. Does kin-biased territorial behavior increase kin-based foraging in juvenile salmonids. *Behavioral Ecology*, **7,** 24-29.

Bush, G. L. 1994. Sympatric speciation in animals: new wine in old bottles. *Trends in Ecology & Evolution*, **9,** 285-288.

Carvalho, G. R. 1993. Evolutionary aspects of fish distribution: genetic variability and adaptation. *Journal of Fish Biology*, **43,** 53-73.

Castiglia, R. & Capanna, E. 2000. Contact zone between chromosomal races of *Mus musculus domesticus*. 2. Fertility and segregation in laboratory-reared and wild mice heterozygous for multiple Robertsonian rearrangements. *Heredity*, **85,** 147-156.

Castric, V., Bernatchez, L., Belkhir, K. & Bonhomme, F. 2002. Heterozygote deficiencies in small lacustrine populations of brook charr *Salvelinus Fontinalis* Mitchill (Pisces, Salmonidae): a test of alternative hypotheses. *Heredity*, **89,** 27-35.

Charlesworth, D. & Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. *Annual Revue of Ecology and Systematics*, **18,** 287-368.

Christensen, B. 1996. Predator foraging capabilities and prey anti-predator behaviours: pre- versus postcapture constraints on size-dependent predator-prey interactions. *Oikos*, **76,** 368-380.

Clement, M., Posada, D. & Crandall, K. A. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9,** 1657-1660.

Clutton-Brock, T. H. & Vincent, A. J. 1991. Sexual selection and the potential reproductive rates of males and females. *Nature*, **351,** 58-60.

Courtenay, S. C., Quinn, T. P., Dupuis, H. M. C., Groot, C. & Larkin, P. A. 1997. Factors affecting the recognition of population-specific odours by juvenile coho salmon. *Journal of Fish Biology*, **50,** 1042-1060.

Coyne, J. A. 1992. Genetics and speciation. *Nature*, **355,** 511-515.

Coyne, J. A. & Orr, H. A. 1989. Patterns of speciation in *Drosophila*. *Evolution*, **43,** 362-381.

Coyne, J. A. & Orr, H. A. 1997. "Patterns of speciation in *Drosophila*" revisited. *Evolution*, **51**, 295-303.

Coyne, J. A. & Orr, H. A. 1998. The evolutionary genetics of speciation. *Philosophical Transactions of the Royal Society Ser. B*, **353,** 287-305.

Cracraft, J. 1983. *Species concepts and speciation analysis*. New York: Plenum Press.

Darwin, C. 1859. *The origin of species by means of natural selection*. London: Murray.

Day, T., Pritchard, J. & Schluter, D. 1994. A comparison of two stickle-

backs. *Evolution*, **48,** 1723-1734.

Dieckmann, U. & Doebeli, M. 1999. On the origin of species by sympatric speciation. *Nature*, **400,** 354-357.

Diehl, S. & Eklöv, P. 1995. Effects Of Piscivore - Mediated Habitat Use On Resources, Diet, and Growth Of Perch. *Ecology*, **76**, 1712-1726.

Dieterich, A. 1998. Die Parasitierung der Flußbarsche (*Perca fluviatilis*) mit Wurmstar und Hechtbandwurm im Bodensee. Diplomarbeit, Limnologisches Institut der Universität Konstanz.

Dobzhansky, T. 1937. *Genetics and the origin of species*. New York: Culumbia University Press.

Dobzhansky, T. 1940. Speciation as a stage in evolutionary divergence. *American Naturalist*, **74,** 312-321.

Doebeli, M. & Dieckmann, U. 2003. Speciation along environmental gradients. *Nature*, **421,** 259-264.

Douglas, M. R., Brunner, P. C. & Bernatchez, L. 1999. Do assemblages of Coregonus (Teleostei: Salmoniformes) in the central alpine region of Europe represent species flocks? *Molecular Ecology*, **8,** 589-603.

Dowling, T. E. & Moore, W. S. 1986. Absence of population subdivision in the common shiner *N. cornutus* (Cyprinidae). *Environmental Biology of Fishes*, **15,** 151-155.

Dowling, T. E. & Secor, C. L. 1997. The role of hybridization and introgression in the diversification of animals. *Annual Revue of Ecology and Systematics*, **28,** 593-619.

Drossel, B. & McKane, A. 2000. Competitive speciation in quantitative genetic models. *Journal of Theoretical Biology*, **204,** 467-478.

Dugatkin, L. A. & Wilson, D. S. 1992. The prerequisites for strategic behaviour in bluegill sunfish *lepomis-macrochirus*. *Animal Behaviour*, **44,** 223-230.

Eklöv, P. 1992. Group foraging versus solitary foraging efficiency in piscivorous predators: the perch, *Perca fluviatilis*, and pike, *Esox lucius*, patterns. *Animal Behaviour*, **44,** 313-326.

Eklöv, P. & Persson, L. 1995. Species-specific antipredator capacities and prey refuges: interactions between piscivorous perch (*Perca fluviatilis*) and juvenile roach (*Rutilus rutilus*). *Behavioral Ecology and Sociobiology*, **37,** 169-178.

Emlen, S. T. & Oring, L. W. 1997. Ecology, sexual selection and the evolution of mating systems. *Science*, **197,** 215-223.

Ferguson, M. M. & Noakes, D. L. G. 1981. Socioal grouping and genetic variation in common shiners, *Notropis cornutus* (Pisces, Cyprinidae). *Environmental Biology of Fishes*, **6,** 357-360.

Fisher, R. A. 1930. *The Genetical Theory of Natural Selection.* Oxford: Clarendon Press.

Fontaine, P. M. & Dodson, J. J. 1999. An analysis of the distribution of juvenile Atlantic salmon (*Salmo salar*) in nature as a function of relatedness using microsatellites. *Molecular Ecology*, **8,** 189-198.

Frommen, J. & Bakker T. C. M. 2004. Adult three-spined sticklebacks prefer to shoal with familiar kin. *Behaviour*, **in press**.

Futuyma, D. 1998. *Evolutionary biology.* Sunderland, Massachusetts: Sinauer.

Garagna, S., Redi, C. A., Zuccotti, M., Britton-Davidian, J. & Winking, H. 1989. Kinetics of oogenesis in mice heterozygous for Robertsonian translocations. *Differentiation*, **42,** 167-171.

Gerlach, G., Schardt, U., Eckmann, R. & Meyer, A. 2001. Kin-structured subpopulations in Eurasian perch (*Perca fluviatilis* L.). *Heredity*, **86,** 213-221.

Gíslason, D., Ferguson M. M., Skúlason, S. & Snorrason S. S. 1999. Rapid and coupled phenotypic and genetic divergence in Icelandic arctic char (*Salvelinus alpinus*). *Canadian Journal of Fisheries & Aquatic Sciences*, **56,** 2229-2234.

Gliwicz, Z. M. & Jachner, A. 1992. Diel migrations of juvenile fish a ghost of predation past or present? *Archiv für Hydrobiologie*, **124,** 385-410.

Godin, J.-G. J. 1986. Antipredator function of shoaling in teleost fishes: a selective review. *Naturaliste canadien*, **113,** 241-250.

Grant, P. R. & Grant, B. R. 1994. Phenotypic and genetic effects of hybridization in Darwin's finches. *Evolution*, **48,** 297-316.

Griffiths, S. W. & Magurran, A. E. 1999. Schooling decisions in guppies (*Poecilia reticulata*) are based on familiarity rather than kin recognition by phenotype matching. *Behavioral Ecology and Sociobiology*, **45,** 437-443.

Gross, R., Kuehn, R., Baars, M., Schroeder, W., Stein, H. & Rottmann, O. 2001. Genetic differentiation of European grayling populations across the Main, Danube and Elbe drainages in Bavaria. *Journal of Fish Biology*, **58,** 264-280.

Hamilton, W. D. 1964. The genetical evolution of social behaviour. *Journal*

of Theoretical Biology, **7,** 1-16.

Hasler, A. D. & Scholz, A. T. 1983. *Olfactory Imprinting and Homing in Salmon*. New York: Springer-Verlag.

Hatfield, T., Barton, N. & Searle, J. B. 1992. A model of a hybrid zone between two chromosomal races of the Common shrew (*Sorex araneus*). *Evolution*, **46,** 1129-1145.

Hatfield, T. & Schluter, D. 1996. A test for sexual selection on hybrids of two sympatric sticklebacks. *Evolution*, **50,** 2429-2434.

Hatfield, T. & Schluter, D. 1999. Ecological speciation in sticklebacks: Environment-dependent hybrid fitness. *Evolution*, **53,** 866-873.

Hauffe, H. C. & Pialek, J. 1997. Evolution of the chromosomal races of *Mus musculus domesticus* in the Rhaetian Alps: the roles of whole-arm reciprocal translocation and zonal raciation. *Biological Journal of the Linnean Society*, **62,** 255-278.

Hauser, L., Carvalho, G. R. & Pitcher, T. J. 1998. Genetic population structure in the Lake Tanganyika sardine *Limnothrissa miodon*. *Journal of Fish Biology, Supplement A*, **53,** 413-429.

Helfman, G. S. 1979. Twilight Activities of Yellow Perch, *Perca flavescens*. *Journal of the Fisheries Research Board of Canada*, **36,** 173-179.

Hewitt, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, **58,** 247-276.

Hewitt, G., M. 2001. Speciation, hybrid zones and phylogeography - or seeing genes in space and time. *Molecular Ecology*, **10,** 537-549.

Higashi, M., Takimoto, G. & Yamamura, N. 1999. Sympatric speciation by sexual selection. *Nature*, **402,** 523-6.

Hjelm, J., Persson, L. & Christensen, B. 2000. Growth, morphological variation and ontogenetic niche shifts in perch (*Perca fluviatilis*) in relation to resource availability. *Oecologia*, **122,** 190-199.

Hjelm, J., Svanback, R., Bystrom, P., Persson, L. & Wahlstrom, E. 2001. Diet-dependent body morphology and ontogenetic reaction norms in Eurasian perch. *Oikos.*, **95,** 311-323.

Hochberg, M. E., Sinervo, B. & Brown, S. P. 2003. Socially mediated speciation. *Evolution*, **57,** 154-158.

Höglund, L. B. 1961. The reaction of fish in concentration gradients. *Report of the Institute of Freshwater Research Drottningholm*, **43,** 1-147.

Höjesjö, J., Johnsson, J. I., Petersson, E. & Järvi, T. 1998. The importance

of being familiar: individual recognition and social behavior in sea trout (*Salmo trutta*). *Behavioral Ecology*, **9**, 445-451.

Johnson, T. C., Scholz, C. A., Talbot, M. R., Kelts, K., Ricketts, R. D., Ngobi, G., Beuning, K., Ssemmanda, I. & McGill, J. W. 1996. Late Pleistocene desiccation of Lake Victoria and rapid evolution of cichlid fishes. *Science*, **273,** 1091-1093.

Kawecki, T. J. 1996. Sympatric speciation driven by beneficial mutations. *Proceedings of the Royal Society of London B*, **263,** 1515-1520.

Kawecki, T. J. 1997. Sympatric speciation by habitat specialization driven by deleterious mutations. *Evolution*, **51,** 1751-1763.

Keller, O. & Krayss, E. 2000. Die Hydrographie des Bodenseeraums in Vergangenheit und Gegenwart. *Berichte der St. Gallischen Naturwissenschaftlichen Gesellschaft*, **89,** 39-56.

Kipling, C. & Le Cren, E. D. 1984. Mark - recapture experiments on fish in Windermere, 1943-1982. *Journal of Fish Biology*, **24,** 395-414.

Kirkpatrick, M. 2000. Reinforcement and divergence under assortative mating. *Proceedings of the Royal Society of London B,* **267,** 1649-1655.

Knowlton, N. 1993. Sibling Species in the Sea. *Annual Revue of Ecology and Systematics*, **24,** 189-216.

Knowlton, N., Maté, J. L., Guzmán, H. M., Rowan, R. & Jara, J. 1997. Direct evidence for reproductive isolation among the three species of the *Montastraea annularis* complex in Central America (Panamá and Honduras). *Marine Biology*, **127,** 705-711.

Kondrashov, A. S. & Shpak, M. 1998. On the origin of species by means of assortative mating. *Proceedings of the Royal Society of London B*, **265,** 2272-2278.

Kondrashov, A. S. & Kondrashov, F. A. 1999. Interactions among quantitative traits in the course of sympatric speciation. *Nature*, **400,** 351-354.

Krause, J., Butlin, R. K., Peuhkuri, N. & Pritchard, V. L. 2000. The social organization of fish shoals: a test of the predictive power of laboratory experiments for the field. *Biological Reviews of the Cambridge Philosophical Society*, **75,** 477-501.

Laland, K. N. 1994. On the evolutionary consequences of sexual imprinting. *Evolution*, **48,** 477-489.

Leclerc, D., Wirth, T. & Bernatchez, L. 2000. Isolation and characterization of microsatellite loci in the yellow perch (*Perca flavescens*), and cross-species amplification within the family Percidae. *Molecular Ecology*, **9,**

993-1011.

Lessios, H. A. & Cunningham, C. W. 1990. Gametic incompatibility between species of the sea urchin *Echinometra* on the two sides of the Isthmus of Panama. *Evolution*, **44,** 933-941.

Lu, G. & Bernatchez, L. 1999. Correlated trophic specialization and genetic divergence in sympatric lake whitefish ecotypes (*Coregonus clupeaformis*): Support for the ecological speciation hypothesis. *Evolution*, **53,** 1491-1505.

Magnhagen, C. & Heibo, E. 2004. Growth in length and in body depth in young-of-the-year perch with different predation risk. *Journal of Fish Biology*, **64,** 612-624.

Magurran, A. E. 1990. The adaptive significance of schooling as an anti-predator defence in fish. *Annales Zoologici Fennici*, **27,** 51-66.

Magurran, A. E. & Pitcher, T. J. 1983. Foraging timidity and shoal size in minnows and goldfish. *Behavioral Ecology and Sociobiology*, **12,** 142-152.

Mathieu, E., Roux, A. M. & Bonhomme, F. 1990. Épreuves de validation dans l'analyse de structures génétiques multivariées: comment tester l'équilibre panmitique? *Revue de Statistique Appliquée*, **38,** 47-66.

Maynard Smith, J. 2001. *Evolutionary Genetics.* New York: Oxford University Press.

Mayr, E. 1942. *Systematics and the origin of species.* New York: Columbia University Press.

Mayr, E. 1963. *Animal species and evolution.* Cambridge, Massachusetts: Harvard University Press.

McPhail, J. D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): morphological and genetic evidence for a species pair in Enos Lake, British Columbia. *Canadian Journal of Zoology*, **62,** 1402-1408.

McPhail, J. D. 1992. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*) evidence for genetically divergent populations in Paxton Lake, Texada Island, British Columbia. *Canadian Journal of Zoology*, **70,** 361-369.

McPhail, J. D. 1993. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): origin of the species pairs. *Canadian Journal of Zoology*, **71,** 515-523.

Melbourne, C., Whiteside, C., Swindoll, M. & Doolittle, W. L. 1985. Factors affecting the early life history of yellow perch, *Perca flavescens*. *Environmental Biology of Fishes*, **12,** 47-56.

Meyer, A. 1993. Phylogenetic relationships and evolutionary processes in East African cichlids. *Trends in Ecology & Evolution*, **8,** 279-284.

Meyer, A., Kocher, T., D., Basasibwaki, P. & Wilson A, C. 1990. Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature*, **347,** 550-553.

Milinski, M. 1987. Tit-for tat in sticklebacks and the evolution of cooperation. *Nature*, **325,** 433-437.

Miller, L. M. 2003. Microsatellite DNA loci reveal genetic structure of Yellow perch in Lake Michigan. *Transactions of the American Fisheries Society*, **132,** 503-513.

Nagel, L. & Schluter, D. 1998. Body size, natural selection, and speciation in sticklebacks. *Evolution*, **52,** 209-218.

Naish, K. A., Carvalho, G. R. & Pitcher, T. J. 1993. The genetic structure and microdistribution of shoals of *Phoxinus phoxinus*, the European minnow. *Journal of Fish Biology, Supplement A*, **43,** 75-89.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89,** 583-590.

Nesbø, C. L., Arab, M. O. & Jakobsen, K. S. 1998a. Heteroplasmy, length and sequence variation in the mtDNA control regions of three percid fish species (*Perca fluviatilis, Acerina cernua, Stizostedion lucioperca*). *Genetics*, **148,** 1907-1919.

Nesbø, C. L., Magnhagen, C. & Jakobsen, K. S. 1998b. Genetic differentiation among stationary and anadromous perch (*Perca fluviatilis*) in the Baltic Sea. *Hereditas*, **129,** 241-249.

Nesbø, C. L., Fossheim, T., Vollestad, L. A. & Jakobsen, K. S. 1999. Genetic divergence and phylogeographic relationships among European perch (*Perca fluviatilis*) populations reflect glacial refugia and postglacial colonization. *Molecular Ecology*, **8,** 1387-1404.

Olsén, K. H. 1986. Chemoattraction between juveniles of two sympatric stocks of Arctic charr (*Salvelinus alpinus* (L.)) and their gene frequency of serum esterases. *Journal of Fish Biology*, **28,** 221-231.

Olsén, K. H. 1989. Sibling recognition in juvenile Arctic charr, *Salvelinus alpinus* (L.). *Journal of Fish Biology*, **34,** 571-581.

Olsén, H. K. 1992. *Kin recognition in fish mediated by chemical cues.* London: Chapman & Hall.

Olsén, K. H., Grahn, M., Lohm, J. & Langefors, A. 1998. MHC and kin discrimination in juvenile Arctic charr, *Salvelinus alpinus* (L.). *Animal*

Behaviour, **56,** 319-327.

Olsén, K. H., Grahn, M. & Lohm, J. 2002. Influence of MHC on sibling discrimination in Arctic charr, *Salvelinus alpinus* (L.). *Journal of Chemical Ecology*, **28,** 21-40.

Osinov, A. G. & Bernatchez, L. 1996. "Atlantic" and "Danube" phylogenetic groups of the sea trout *Salmo trutta* complex: Genetic divergence, evolution, protection. *Voprosy Ikhtiologii*, **36,** 762-786.

Palumbi, S. R. 1994. Genetic Divergence, Reproductive Isolation and Marine Speciation. *Annual Revue of Ecology and Systematics*, **25,** 547-572.

Peuhkuri, N. & Seppae, P. 1998. Do three-spined sticklebacks group with kin? *Annales Zoologici Fennici*, **35,** 21-27.

Pitcher, T. J., Magurran, A. E. & Winfield, I. 1982. Fish in larger shoals find food faster. *Behavioral Ecology and Sociobiology*, **10,** 149-151.

Pitcher, T. J. & Parrish, J. K. 1993. Functions of shoaling behaviour in teleosts. In: *Behaviour of teleost fishes* T. J. Pitcher (ed). pp. 363-439. London: Chapman & Hall.

Pouyaud, L., Desmarais, E., Chenuil, A., Agnese, J. F. & Bonhomme, F. 1999. Kin cohesiveness and possible inbreeding in the mouthbrooding tilapia *Sarotherodon melanotheron* (Pisces Cichlidae). *Molecular Ecology*, **8,** 803-812.

Quinn, T. P. & Busack, C. A. 1985. Chemosensory recognition of siblings in juvenile coho salmon (*Oncorhynchus kisutch*). *Animal Behaviour*, **33,** 51-56.

Quinn, T. P. & Hara, T. J. 1986. Sibling recognition and olfactory sensitivity in juvenile coho salmon. *Canadian Journal of Zoology*, **64,** 921-925.

Redi, C. A., Garagna, S., Hilscher, B. & Winking, H. 1985. The effects of some Robertsonian chromosome combinations in the seminiferous epithelium of the mouse. *Journal of Embryology and Experimental Morphology*, **85,** 1-19.

Refseth, U. H., Nesbø, C. L., Stacy, J. E., Voellestad, L. A., Fjeld, E. & Jakobsen, K. S. 1998. Genetic evidence for different migration routes of freshwater fish into Norway revealed by analysis of current perch (*Perca fluviatilis*) populations in Scandinavia. *Molecular Ecology*, **7,** 1015-1027.

Reusch, T. B. H., Häberli, M. A., Aeschlimann, P. B. & Milinski, M. 2001. Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature*, **414,** 300-302.

Rice, W. R. & Hostert, E. E. 1993. Laboratory experiments on speciation:

what have we learned in 40 years? *Evolution*, **47,** 1637-1653.

Ridgway, M. S. & McPhail, J. D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): mate choice and reproductive isolation in the Enos Lake species pair. *Canadian Journal of Zoology*, **62,** 1813-1818.

Riffel, M. & Schreiber, A. 1998. Morphometric differentiation in populations of the Central European sculpin *Cottus gobio* L., a fish with deeply divergent genetic lineages. *Canadian Journal of Zoology*, **76,** 876-885.

Robinson, B. W. & Wilson, D. S. 1996. Genetic variation and phenotypic plasticity in a polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*). *Evolutionary Ecology*, **10,** 631-652.

Robinson, B. W. & Parsons, K. J. 2002. Changing times, spaces, and faces: tests and implications of adaptive morphological plasticity in the fishes of northern postglacial lakes. *Canadian Journal of Fisheries & Aquatic Sciences*, **59,** 1819-1833.

Rundle, H. D. & Schluter, D. 1998. Reinforcement of stickleback mate preferences: Sympatry breeds contempt. *Evolution*, **52,** 200-208.

Ruzzante, D. E., Walde, S. J., Cussac, V. E., Macchi, P. J. & Alonso, M. F. 1998. Trophic polymorphism, habitat and diet segregation in *Percichthys trucha* (Pisces: Percichthyidae) in the Andes. *Biological Journal of the Linnean Society*, **65,** 191-214.

Said, K., Saad, A., Auffray, J.-C. & Britton-Davidian, J. 1993. Fertility estimates in the Tunisian all-acrocentric and Robertsonian populations of the house mouse and their chromosomal hybrids. *Heredity*, **71,** 532-538.

Sambrook, J., Fritsch, E. F. & Maniatis, T. 1989. *Molecular Cloning: a Laboratory Manual.* New York, Cold Spring Harbor: Laboratory Press.

Schliewen, U. K., Tautz, D. & Paabo, S. 1994. Sympatric speciation suggested by monophyly of crater lake cichlids. *Nature*, **368,** 629-632.

Schliewen, U., Rassmann, K., Markmann, M., Markert, J., Kocher, T. & Tautz, D. 2001. Genetic and ecological divergence of a monophyletic cichlid species pair under fully sympatric conditions in Lake Ejagham, Cameroon. *Molecular Ecology*, **10,** 1471-1488.

Schluter, D. 1994. Experimental evidence that competition promotes divergence in adaptive radiation. *Science*, **266,** 798-801.

Schluter, D. 1995. Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. *Ecology*, **76,** 82-90.

Schluter, D. 1996. Ecological causes of adaptive radiation. *American Naturalist*, **148** (supplement)**,** 40-64.

Schluter, D. 1998. Ecological causes of speciation. In: *Endless forms: species and speciation* Howard, D. J. & Berlocher, S. H (ed). pp. 114-129. Oxford U.K.: Oxford University Press.

Schluter, D. 2001. Ecology and the origin of species. *Trends in Ecology & Evolution*, **16,** 372-380.

Schluter, D. & McPhail, J. D. 1992. Ecological character displacement and speciation in sticklebacks. *American Naturalist*, **140,** 85-108.

Schneider, S., Roessli, D. & Excoffier, L. 2000. Arlequin ver. 2.000: A software for population genetics data analysis. In: *Genetics and Biometry Laboratory*. Switzerland: University of Geneva.

Seehausen, O. & Van Alphen, J. J. M. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science*, **277,** 1808-1811.

Seehausen, O. & Van Alphen, J. J. M. 1998. The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei* complex). *Behavioral Ecology and Sociobiology*, **42,** 1-8.

Seehausen, O. & van Alphen, J. J. M. 1999. Can sympatric speciation by disruptive sexual selection explain rapid evolution of cichlid diversity in Lake Victoria? *Ecology Letters*, **2,** 262-271.

Seger, J. 1991. *Cooperation and conflict in social insects.* Oxford: Blackwell Scientific Publications.

Skúlason, S. & Smith, T. B. 1995. Resource polymorphism in vertebrates. *Trends in Ecology & Evolution*, **10,** 366-370.

Skúlason, S., Snorrason S. S. & Jónsson, B. 1999. *Sympatric morphs, populations and speciation in freshwater fish with emphasis on Arctic charr*. Oxford U. K.: Oxford University Press.

Smith, R. J. F. 1982. The adaptive significance of the alarm substance-fright reaction system. In: *Chemoreception in Fishes* Hara, T. J.(ed). pp. 327-342. Amsterdam: Elsevier.

Smith, R. J. F. 1986. *The evolution of chemical alarm signals in fishes*. New York: Plenum Press.

Smith, T. B. & Skúlason, S. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. *Annual Revue of Ecology and Systematics*, **27,** 111-133.

Stabell, O. B. 1982. Detection of natural odorants by Atlantic salmon parr using positive rheotaxis olfactometry. In: *Proc. Salmon and Trout*

Migratory Behavior Symp. Brannon, E. L. & Salo, E. O. (ed). pp. 71-78. Seattle: Univ.Washington Press.

Stabell, O. B. 1987. Intraspecific pheromone discrimination and substrate marking by Atlantic salmon parr. *Journal of Chemical Ecology*, **13,** 1625-1643.

Stearns, S. C. & Hoekstra, R. F. 2001. *Evolution an introduction.* New York: Oxford University Press.

Steck, N., Wedekind, C. & Milinski, M. 1999. No sibling odor preference in juvenile threespined sticklebacks. *Behavioral Ecology*, **10,** 493-497.

Strathmann, R. R. 1981. On the barriers to hybridization between *Srongylocentrotus droebachiensis* (O.F. Muller) and *S. pallidus* (G. O. Sars). *Journal of Experimental Marine Biology and Ecology*, **55,** 39-47.

Street, N. G. & Hart, P. J. B. 1985. Group size and patch location by the stoneloach, *Noemacheilus barbatulus*, a non-visually foraging predator. *Journal of Fish Biology*, **217,** 785-792.

Taberlet, P., Fumagalli, L., Wust-Saucy, A.-G. & Cosson, J.-F. 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, **7,** 453-464.

Tang-Martinez, Z. 2001. The mechanisms of kin discrimination and the evolution of kin recognition in vertebrates: A critical re-evaluation. *Behavioural Processes*, **53,** 21-40.

Templeton, A. R., Crandall, K. A. & Sing, C. F. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data: III. Cladogram estimation. *Genetics*, **132,** 619-633.

Thorpe, J. E. 1977. Morphology, physiology, behavior, and ecology of *Perca fluviatilis* L. and *P. flavescens* Mitchill. *Journal of the Fisheries Research Board of Canada*, **34,** 1504-1514.

Tregenza, T. & Butlin, R. K. 1999. Speciation without isolation. *Nature*, **400,** 311-312.

Trivers, R. L. 1972. Parental Investment and sexual selection. In: *Sexual Selection and the Descent of Man.* Campbell, D. (ed). pp. 139-179. Chicago: Aldine.

Turelli, M. 2001. Theory and speciation. *Trends in Ecology & Evolution*, **16,** 330-343.

Turner, G. F. & Burrows, M. T. 1995. A model of sympatric speciation by sexual selection. *Proceedings of the Royal Society of London B*, **260,**

287-292.

Uehara, T. & Shingaki, M. 1984. Studies on the fertilization and development in the two types of *Echinometra mathaei* from Okinawa. *Zoological Science*, **1,** 1008.

Vamosi, S., M. & Schluter, D. 1999. Sexual selection against hybrids between sympatric stickleback species: Evidence from a field experiment. *Evolution*, **53,** 874-879.

Van Havre, N. & Fitzgerald, G. J. 1988. Shoaling and kin recognition in the threespine Stickleback (*Gasterosteus aculeatus* L.). *Biology of Behaviour*, **13,** 190-201.

Via, S. 2001. Sympatric speciation in animals: the ugly duckling grows up. *Trends in Ecology & Evolution*, **16,** 381-390.

Wainwright, P. C. & Richard, B. A. 1995. Predicting patterns of prey use from morphology of fishes. *Environmental Biology of Fishes*, **44,** 97-113.

Wang, N. & Appenzeller, A. 1998. Abundance, depth distribution, diet composition and growth of perch (*Perca fluviatilis*) and burbot (*Lota lota*) larvae and juveniles in the pelagic zone of Lake Constance. *Ecology of Freshwater Fish*, **7,** 176-183.

Wang, N. & Eckmann, R. 1994. Distribution of perch (*Perca fluviatilis* L.) during their first year of life in Lake Constance. *Hydrobiologia*, **277,** 135-143.

Warburton, K. & Lees, N. 1996. Species discrimination in guppies: learned responses to visual cues. *Animal Behaviour*, **52,** 371-378.

Weiss, S., Persat, H., Eppe, R., Schlötterer, C. & Uiblein, F. 2002. Complex patterns of colonization and refugia revealed for European grayling *Thymallus thymallus*, based on complete sequencing of the mitochondrial DNA control region. *Molecular Ecology*, **11,** 1393-1407.

Weiss, S., Schlötterer, C., Waidbacher, H. & Jungwirth, M. 2001. Haplotype (mtDNA) diversity of brown trout *Salmo trutta* in tributaries of the Austrian Danube: Massive introgression of Atlantic basin fish: By man or nature? *Molecular Ecology*, **10,** 1241-1246.

West-Eberhard, M. J. 1983. Sexual Selection, Competition and Speciation. *The Quarterly Review of Biology*, **58,** 155-183.

Zahavi, A. 1975. Mate selection - A selection for a handicap. *Journal of Theoretical Biology*, **53,** 205-214.

Chapter 9

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