First publ. in: Behavioral Ecology and Sociobiology 59 (2006), 4, pp. 461-468

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Kin and population recognition in sympatric Lake Constance perch (*Perca fluviatilis* L.): can assortative shoaling drive population divergence?

Abstract Prior studies have shown that perch (*Perca fluviatilis* L.) of Lake Constance belong to two genetically different but sympatric populations and that local aggregations of juveniles and adults contain closely related kin. In this study, we analysed the genetic structure of pelagic perch larvae to investigate if kin-structured shoals already exist during early ontogenetic development or might be the result of homing to natal sites. Analysis of the gene frequencies at five microsatellite loci revealed that three out of five pelagic aggregations of larvae showed significant accumulation of kin. To investigate possible mechanisms of shoal formation, we tested if perch use olfactory cues to recognize their kin. Choice tests in a fluviarium showed preference for odours of unfamiliar kin vs unfamiliar non-kin. Additionally, we showed that perch could differentiate between the odours of the two sympatric populations and significantly preferred unfamiliar and unrelated conspecifics of their own over the foreign population. Our results present a behavioural mechanism that can lead to the observed formation of kin-structured shoals in perch. We further discuss if the ability to discriminate between their own and a foreign population can result in assortative mating within populations and thus form the basis of "socially mediated speciation" in perch.

Keywords Kin recognition · Population recognition · Kin structure · Microsatellites · Relatedness

Communicated by J. Krause

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Introduction

The formation of fish shoals is a very common phenomenon and can be found throughout numerous freshwater and marine species. These shoals are often strongly assorted by species, body size and/or colour (reviewed in Krause et al. 2000a,b). Additionally, a preference to shoal with familiar conspecifics has been shown for a variety of freshwater fish such as guppies, *Poecilia reticulata* (Griffiths and Magurran 1999), bluegill sunfish, *Lepomis macrochirus* (Dugatkin and Wilson 1992) three-spined sticklebacks, *Gasterosteus aculeatus* (Van Havre and Fitzgerald 1988; Barber and Ruxton 2000) and brown trout, *Salmo trutta* (Höjesjö et al. 1998). But only very little is known about the relationship of shoaling and the genetic structure of natural fish populations. Shoaling with kin can be advantageous; closely related animals are more likely to show cooperative behaviour during risky situations such as predator inspection (Milinski 1987) because helping a relative can increase the indirect fitness of an individual (Hamilton 1964). Several authors studied relatedness between shoal members using allozymes (Ferguson and Noakes 1981; Avise and Shapiro 1986; Dowling and Moore 1986; Naish et al. 1993; Hauser et al. 1998; Peuhkuri and Seppae 1998) or microsatellite markers (Pouyaud et al. 1999). The results were very ambiguous; no indication for kin-structured shoals was found for *Anthias squamipinnis* (Avise and Shapiro 1986), common shiner, *Notropis cournutus* (Dowling and Moore 1986), European minnow, *Phoxinus phoxinus* (Naish et al. 1993), Tanganyikan sardine, *Limnonthrissa miodon* (Hauser et al. 1998) and three-spined stickleback (Peuhkuri and Seppae 1998), while Ferguson and Noakes (1981) indicated that kin structure in the common shiner might exist. Only Pouyaud et al. (1999) found significant association of kin in shoals of the mouthbrooding tilapia, *Sarotherodon melanotheron*.

A preference to shoal with kin would imply some kind of kin recognition mechanism that allows for the differentiation of related conspecifics from non-kin. There are two main types of kin recognition, direct familiarity and indirect familiarity (phenotype matching). In the case of

direct familiarity, individuals become familiar with members of the same litter or clutch during early ontogeny and prefer these individuals over unfamiliar ones (Olsén 1992). In the case of phenotype matching, an individual learns about the phenotype of close relatives or itself (self-matching) during early development and later compares unfamiliar conspecifics with this learned phenotype to identify kin (Olsén 1992; Sherman et al. 1997). Phenotype matching implies a correlation of the phenotypic traits used for kin recognition and the genotype because only heritable phenotypic traits can be true indicators for relatedness (Tang-Martinez 2001).

Kin recognition has been documented for several salmonid species such as Arctic charr, *Salvelinus alpinus* (Olsén 1989), Atlantic salmon, *Salmo salar*, rainbow trout, *Oncorhynchus mykiss* (Brown and Brown 1992), rainbowfish, *Melanotaenia eachamensis* (Arnold 2000) and zebrafish, *Danio rerio* (Mann et al. 2003). Contradictory results were obtained for coho salmon, *Oncorhynchus kisutch* (Quinn and Busack 1985; but see Quinn and Hara 1986), for sticklebacks (Van Havre and Fitzgerald 1988; Frommen and Bakker 2004; but see Steck et al. 1999) and for the Trinidadian guppy, *Poecilia reticualta* (Warburton and Lees 1996; but see Griffiths and Magurran 1999).

However, shoaling with relatives can also be disadvantageous through the risk of inbreeding if related individuals mate. Inbreeding can increase the genetic homozygosity and, thus, the possible expression of recessive deleterious mutations in offspring (Charlesworth and Charlesworth 1987). Mechanisms to recognize kin can therefore be advantageous in two ways: an individual may profit from the benefits of cooperation with relatives, and it can minimise inbreeding depression by avoiding to mate with siblings. Griffiths and Armstrong (2001) have shown, however, that for territorial animals kin associations can also be disadvantageous.

On the other hand, outcrossing of individuals from different populations can be disadvantageous as well due to a break-up of coadapted gene complexes or favourable epistatic relationships that have developed as an adaptation to different habitat parameters (Mayr 1963; Shields 1982). Thus, with ongoing population divergence, selection should favour mechanisms that lead to a shift from outbreeding enhancement to outbreeding depression (Lynch 1991). For organisms belonging to sympatric populations, the recognition of population-specific cues provides an excellent mechanism to recognize appropriate mating partners of their own population. Species living in highly structured, dark or turbid habitats might evolve the ability to use non-visual features, such as acoustic or olfactory (chemical) cues, to discriminate between populations. Closely related species of Hawaiian crickets find their mating partners based on calling song differences, suggesting that acoustic features could have been the first cues to change during the speciation process (reviewed in Shaw and Herlihy 2000). For the songbird greenish warbler (*Phylloscopus trochiloides*), Irwin et al. (2001) showed that sexual selection for increased complexity of song is driving population divergence. Unlike kin recognition, olfactory-based population recognition in fish has, to our knowledge, only been studied in salmonids (for a review, see Olsén 1992). Evidence for population recognition based on chemical cues was provided for Atlantic salmon (Stabell 1982, 1987), Arctic charr (Olsén 1986) and coho salmon (Courtenay et al. 1997). Recently, Ward and Krause (2004) have shown that chemical cues play a major role in the recognition of familiar conspecifics but can be influenced by different habitat and diet experiences.

This study focuses on Eurasian perch (*Perca fluviatilis* L.). During their ontogeny, perch of Lake Constance show a typical habitat shift. Soon after hatching, larvae move to the pelagic zone to feed on zooplankton and return about 1 month later to the littoral (Wang and Eckmann 1994). As juveniles and adults, perch remain in the littoral during summer or in close proximity to the littoral when they move to deeper waters during winter (Wang and Appenzeller 1998). For perch of Lake Constance, pairwise calculations of relatedness provided evidence that aggregations of juveniles and adults caught in the littoral during summer were genetically structured, containing closely related kin within age groups (Gerlach et al. 2001). This observed kin structure could be the result of homing of pelagic larvae to natal sites (Aalto and Newsome 1989), or it could be based on kin preference. Calculating the corrected genetic index G_{ST} of between-population divergence for perch of Lake Constance, Gerlach et al.(2001) found a subdivision into two populations with restricted gene flow (G_{ST}=0.07). One population was found to inhabit the eastern part, and the other population inhabited the western part of the lake. No obvious geographical barrier separated the two.

Thus, the aim of our study was twofold. Firstly, we investigated whether kin aggregations like those observed in juveniles and adults are already present in free-ranging shoals of larval perch. Odour choice tests in the laboratory were conducted to test whether the kin-structured perch aggregations might be based on olfactory kin recognition and kin preference. Secondly, we tested whether perch of the two sympatric populations also use olfactory recognition to distinguish between conspecifics from the same population vs a foreign one.

Methods

Microsatellite genotyping

Perch larvae were sampled on one occasion from the surface in the pelagic zone of Lower Lake Constance with a horizontally trawled plankton net (0.25-m^2 opening). We used a "multi-net", consisting of five conical nets of the same size that could be opened and closed individually one after the other. Thus, during the sampling haul of 722 m, each net covered a sampling transect of approximately 140 m in length.

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 loci isolated from walleye, *Stizostedion vitreum* (*SVI* 6, 17 and 18; GenBank accession nos. G36962–64; Borer et al. 1999) and two from yellow perch, *Perca flavescens* (*PF* 1 and 5; GenBank accession nos. AF211826–30, Leclerc et al. 2000). A summary of the loci characteristics is presented in Table 1. The PCR products were run individually on Spreadex gels (Elchrom Scientific AG, Switzerland; EL 400 for *SVI* 6 and 17 and *PF* 1 and 5; EL 600 for *SVI* 18) using the SEA 2000 advanced submerged gel electrophoresis apparatus (Elchrom). 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).

Observed and expected values for heterozygosity were determined using the GENETIX404 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 multi-locus genotypic data (for details, see Belkhir et al. 2002). To detect relatedness within distinct groups (aggregations of perch) belonging to the same population, the statistics had to be extended (K. Belkhir, personal communication). To test if larvae within one sample were genetically more related than expected under random distribution, the mean identity index of all perch pairs within one sample was compared with the null distribution of no relatedness. As null distribution, we calculated the distribution of identity indices of randomly generated subsamples of the same sample size (20 individuals). Subsamples were generated by random permutation of genotypes in 1,000 randomized subsamples, using all five larvae samples as genotype pool. The statistical procedure of testing for significance was the same as described in Castric et al. (2002).

Kin and population recognition

During spawning time, perch were captured with gill nets. To obtain full sibs, the egg strand of a ripe female was cut into pieces, which were then individually fertilised with the sperm of the same male. For maternal half-sibs, the egg strand of one female was cut into pieces, which were then fertilised each by a different male. For paternal half-sibs, one male was used to fertilise pieces of egg strands of different females. All 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°C; 14-h illumination). Thus, each aquarium contained a group of full sibs. After hatching, temperature was raised to 20°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 were carried out with fish between 3 and 6 months of age (6–9 cm total length, 2–6 g wet mass).

The odour choice tests were carried out in a two-choice fluviarium (Höglund 1961; Steck et al. 1999; Atema et al. 2002; Fig. 1). 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) 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 (33×25 cm) was separated by screens from the other compartments. A slow but permanent flow-through of tap water (4 l min^{-1}, velocity 0.6 cm s^{-1}, temperature 20°C) ensured that no chemical information about perch was initially present in the flume or was accumulating in the system during the trials. Stimulus water was taken from the holding tanks of the respective stimulus group and was added (7.6 ml min^{-1}) to either side of the inlet compartment using a peristaltic pump. 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 videotaped for later analysis.

Since single test fish had shown erratic behaviour and had no food intake during preliminary trials, we used a small group of four full sibs out of the same holding tank for the choice experiments in the flume. Acclimation time was between 1 and 8 days, during which the fish were fed daily. We defined acclimation to be completed when the fish started feeding directly after food was supplied. Four different trials were run, with consecutive trials separated by at least 30 min recovery time when no odour stimulus was supplied. The following trials (1–4) were run with the order of trials set at random: (1) unfamiliar full sibs vs

Table 1 Microsatellite characteristics

Locus	No. of alleles	Size range (bp)	H_{obs}	H_{exp}	Primer sequences
SVI $18_{(AC)}$	6	160–174	0.616	0.546	GATCTGTAAACTCCAGCGTGCTTAAGCTGCTCAGCATCCAGG
SVI $17_{(AC)}$	6	110–142	0.475	0.550	GCGCACTCTCGCATAGGCCCTGCGTTAAAGTCCTTGGAAACC
SVI $6_{(AC)}$	5	106–120	0.667	0.616	CATATTATGTAGAGTGCAGACCCTGAGCTTCACCTCATATTCC
PF $1_{(GA)}$	12	112–140	0.687	0.737	AAGCAGCCTGATTATATATCCAGACAATTAAACATGCAAC
PF $5_{(GT)}$	6	134–152	0.404	0.471	TGAGAGCCCATGAATTACGCAAACACAGCCAATTTAG

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 1987)

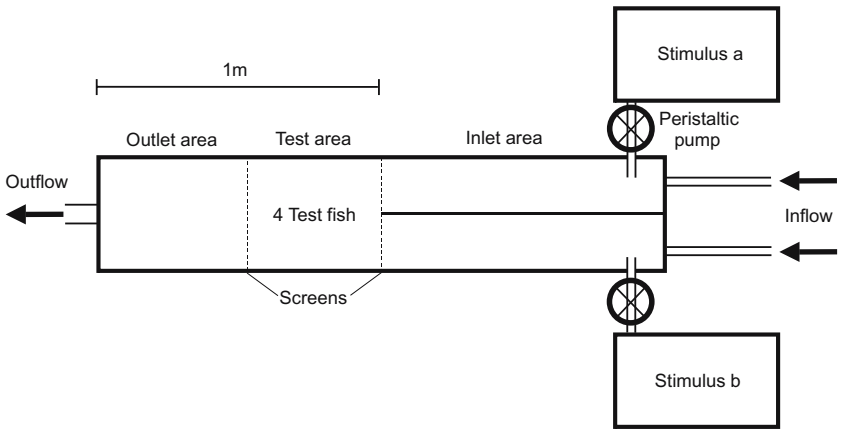

Fig. 1 Experimental choice fluviarium for odour preference tests with perch

unfamiliar non-kin, n=14; (2) unfamiliar maternal half-sibs vs unfamiliar non-kin, n=10; (3) unfamiliar paternal half-sibs vs unfamiliar non-kin, n=9; and (4) unfamiliar and unrelated perch from their own population vs unfamiliar perch from a foreign population, n=13. To account for potential side bias of the fish, the odour stimuli were alternated during trials between the two sides of the flume and were presented two times for 3 min on each side. The number of fish on either side of the test area (corresponding to the two odour stimuli) was counted every 5 s during a 3-min odour supply. After the last trial, all four test fish were removed from the flume and excluded from further experiments.

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 analysis of variance (ANOVA, Jmp vers. 4.0) was used to test for differences between the different kin-recognition trials (full sibs, maternal or paternal half-sibs vs non-kin). Since there were no significant differences, the data was pooled for further analysis. A two-tailed paired t test (Jmp vers. 4.0) was used to compare the average number of fish on each of the two stimulus sides (kin vs non-kin or own vs foreign population).

Results

Microsatellite genotyping

We caught 404 perch larvae (mean, 80 larvae per sample) within the pelagic zone of Lower Lake Constance. 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 1).

The I_{xy} values of randomly generated pairs (subsamples of 20 individuals) are shown as null distribution in Fig. 2 together with the mean I_{xy} values for each larvae sample. The mean pairwise identity index in three of the five net samples departed significantly from the null expectation of no relatedness (Fig. 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).

Kin and population recognition

Perch showed a clear reaction to odour supply of stimulus water from conspecifics. Approximately 1–2 min after the beginning of odour supply (odour plume needs 1 min to reach the downstream end of the test area), fish became active, swimming upstream and pressing their snouts against the screen. 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 stimulus preference.

Some groups of fish, although swimming actively, never changed sides during a trial and were therefore not able to choose between odour stimuli. Additionally, some fish were completely inactive during a trial (fish were resting on the bottom) and thus they were not able to choose between different stimuli either. To eliminate such time intervals, we calculated an "activity index" for each of the 3 min of a trial. We divided the observed number of changes between the two sides of the test area during 1 min of odour supply

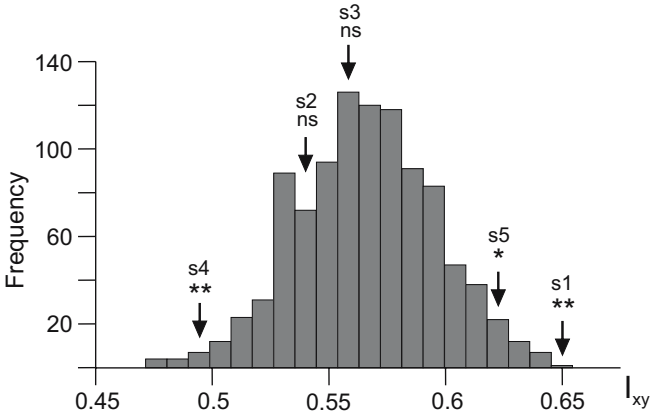

Fig. 2 Frequency distribution of coefficients of relatedness (pairwise identity index calculated by the IDENTIX computer package (Belkhir et al. 2002). The distribution was calculated (1,000 randomizations) based on the allele frequencies of larvae caught in the pelagic zone of Lake Constance, assuming random association of mono-locus genotypes. *Arrows* indicate observed values of relatedness in five samples (*s1–s5*) of perch larvae (for details, see text)

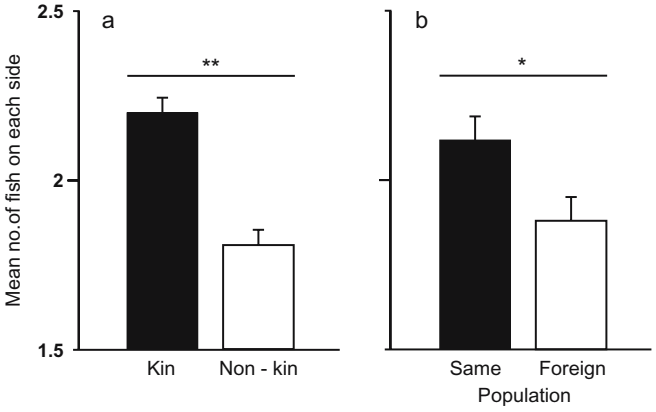

Fig. 3 Outcome of odour choice tests in a fluviarium. Groups of four test fish (full sibs of the same holding tank) were given the choice between different odour stimuli. As a measure for stimulus preference, the mean number of fish (error bars, SE) on the side where the distinct stimulus was added is presented. **a** *Filled bar*, kin odour; *open bar*, non-kin odour. **b** *Filled bar*, odour of the same population; *open bar*, odour of a foreign population

by the maximal number of side changes that are possible (four fish×twelve 5-s intervals = 48 side changes possible). Minutes with activity indices lower then 0.1 (i.e. less than 10% of the maximal number of side changes were observed) were eliminated. This procedure resulted in unequal sample sizes for different stimulus trials.

Given that no significant difference in preference could be seen between the three different kin recognition tests (ANOVA, among all categories of different sibships $F_{2,30}$=2.3, p=0.139), all kin data were pooled. Perch showed a significant preference for their siblings vs non-kin (two-tailed paired t test, t=3.00, n=33, p=0.005; Fig. 3a).

Given the choice between fish of the same vs the foreign population, perch significantly preferred conspecifics of the own population (two-tailed paired t test, t=2.36, n=13, p=0.04; Fig. 3b).

Discussion

Microsatellite genotyping

Our genetic data showed that larval perch stayed together in kin groups. Two out of five larvae samples that were collected in close proximity shared more alleles, 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 the shoal consists of only one family, the number of shared alleles between individuals would be higher than under random distribution of individuals. However, if the shoal consists of members of several families that are less related than any families taken randomly from the population, relatedness can be significantly lower than under random distribution. (L. Bernatchez and V. Castric, personal communication).

Our sampling method did not allow the identification of distinct larval shoals. Thus, a sampling of two or more 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 (Gerlach et al. 2001). Therefore, we conclude that related perch stay together throughout their early development and later in life. Thus, homing of perch to their natal sites, although it could occur, is not the exclusive cause for kin structure in age groups.

Kin and population recognition

We showed that a fish species that 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 showed contradictory results. Brown and Brown (1992, 1993, 1996) showed in numerous laboratory experiments that salmonids, including Atlantic salmon, can discriminate kin and show kin-biased social behaviour by gaining benefits in higher growth rates and lesser aggressive interactions with siblings as territory neighbours. However, a field study of Atlantic salmon larvae and fry in their natural habitat showed that although numerous fish were closely related, as shown by microsatellite analysis, relatives did not preferentially occupy neighbouring territories (Fontaine and Dodson 1999). Griffiths and Armstrong (2001) showed that a heterogeneous advantage could outweigh the benefits of kin-biased behaviour for Atlantic salmon in its natural habitat.

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

Our genetic analysis on the relatedness of free-ranging perch larvae clearly shows that even during the mobile pelagic phase, kin structure of shoals is maintained. Aggregations of newly hatched perch can be transported passively in the water column. In this case, related fish would stay together, independent of any preference for distinct conspecifics. However, with ongoing ontogenetic development during the pelagic phase, perch grow to be active swimmers and aggregate in free-ranging shoals (personal observations). So far, the kin structure in perch shoals could still be the result of a preference to shoal with familiar fish. However, our olfactory test shows that kin

recognition is not based on familiarity since even unfamiliar sibs were preferred over unfamiliar unrelated fish.

Helfman (1984) investigated shoaling behaviour in adult yellow perch (12–20 cm total length) of Cazenovia Lake, Madison County, New York, and detected low school fidelity in this species. He argues that under high predator pressure, shoal fidelity may be part of the anti-predator function of schooling and can result in strong association of individual fish. Under weak predator pressure, however, other functions of schooling, e.g. increased foraging efficiency, could lead to decreased importance of shoaling with familiar or related individuals and thus would lead to weak associations between individual fish. Larval perch, in contrast to adult yellow perch in Cazenovia Lake, are under much higher predation pressure. Under these conditions, high shoal fidelity in Lake Constance perch and even association with familiar or related individuals could have evolved as an anti-predator function based on mutualistic or even altruistic acts directed towards kin.

Olfactory recognition and preference in perch is based on phenotype matching learned from related individuals (Brown et al. 1993; Tang-Martinez 2001). Variability of major histocompatibility complex (MHC) genes might be involved in these recognition processes (Bernatchez and Landry 2003). Female sticklebacks differentiated between males according to sequence differences of their MHC alleles (Reusch et al. 2001), and juvenile Arctic charr chose the water scented by fish with their own MHC type (Olsén et al. 1998, 2002).

In this study, we showed for two sympatric perch populations that juveniles of one population could recognize their own population vs a foreign one by water-borne chemical cues. Similar to some terrestrial species that use acoustic features as non-visual cues to discriminate between populations (Irwin et al. 2001; Shaw and Herlihy 2000), aquatic species in particular use chemical cues as appropriate means for population discrimination. Most experiments on population recognition in fish have been done with salmonids. Migrating salmonid species learn about the odour of their natal rivers as juveniles and use it for orientation when they return as adults to spawn in freshwater rivers (reviewed by Hasler and Scholz 1983). However, it has also been hypothesized that salmonids are guided to their natal rivers by population-specific odours of juveniles. Preference tests in the laboratory showed that salmonids can recognize population-specific cues and mostly prefer the odour of the same over a foreign population (reviewed in Olsén 1992). We showed that juvenile perch prefer odours of their own population vs a foreign one that lives in sympatry within the same lake. The ability of juvenile perch to discriminate between their own population and a foreign one can be based on olfactory imprinting on population-specific cues that can be used later in life for mate-choice decisions. This has also been suggested for salmon fry (Courtenay et al. 1997). Further experiments are necessary to test whether perch mate assortatively within populations and prefer mating partners of their own population over a foreign one, as our genetic data indicate.

We have shown that perch use olfactory cues in another important context, i.e. for kin discrimination. We hypothesize that kin recognition is the basal mechanism from which olfactory-based population recognition was derived. It is conceivable that the specific allelic composition of MHC genes, which are known to influence the individual body odour (Apanius et al. 1997), are involved in both cases. In this scenario, individuals would recognize family-specific MHC alleles, as has been shown for house mice (for a review, see Penn and Potts 1999) and also for Arctic charr (Olsén et al. 1998, 2002), as well as population-specific MHC alleles. Thus, during mate choice, olfactory discrimination of adult perch can lead to assortative mating within populations and avoid inbreeding at the same time. This would be a perfect mechanism to reinforce reproductive isolation between the two sympatric populations.

By combining behavioural and genetic data, we present a possible mechanism for the observed maintenance of the two genetically different populations of perch in Lake Constance. We believe that ours is the first empirical study suggesting that social preferences might drive population divergence.

Acknowledgements We thank Louis Bernatchez, Vincent Castric and Jelle Atema for discussion and comments and Khalid Belkhir for providing a special version of IDENTIX to calculate relatedness in subsamples. We further thank two anonymous referees whose comments greatly improved the manuscript. Funding was provided by Deutsche Forschungsgemeinschaft within the collaborative research centre SFB 454, Littoral of Lake Constance, the "Fonds der Chemischen Industrie", the University of Konstanz, and the "Konrad Adenauer-Stiftung". We declare that the experiments we have presented here comply with the current laws of Germany.

References

Aalto SK, Newsome GE (1989) Evidence of demic structure for a population of yellow perch (*Perca flavescens*). Can J Fish Aquat Sci 46:184–190

Apanius V, Penn D, Slev PR, Ruff LR, Potts WK (1997) The nature of selection on the major histocompatibility complex. Crit Rev Immunol 17:179–224

Arnold KE (2000) Kin recognition in rainbowfish (*Melanotaenia eachamensis*): sex, sibs and shoaling. Behav Ecol Sociobiol 48:385–391

Atema J, Kingsford M, Gerlach G (2002) Larval reef fish could use odour for detection, retention and orientation to reefs. Mar Ecol Prog Ser 241:151–160

Avise JC, Shapiro DY (1986) Evaluating kinship of newly settled juveniles within social groups of the coral reef fish *Anthias squamipinnis*. Evolution 40:1051–1059

Barber I, Ruxton GD (2000) The importance of stable schooling: do familiar sticklebacks stick together? Proc R Soc Lond B Biol Sci 267:151–155

Barnett C (1986) Rearing conditions affect chemosensory preferences in young cichlid fish. Ethology 72:227–235

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

Belkhir K, Castric V, Bonhomme F (2002) IDENTIX, a software to test for relatedness in a population using permutation methods. Mol Ecol Notes 2:611–614

Bernatchez L, Landry C (2003) MHC studies in nonmodel vertebrates: what have we learned about natural selection in 15 years? J Evol Biol 16:363–377
Borer SO, Miller LM, Kapuscinski AR (1999) Microsatellites in walleye *Stizostedion vitreum*. Mol Ecol 8:336–338
Brown GE, Brown JA (1992) Do rainbow trout and Atlantic salmon discriminate kin? Can J Zool 70:1636–1640
Brown GE, Brown JA (1993) Do kin always make better neighbours? The effects of territory quality. Behav Ecol Sociobiol 33:225–231
Brown GE, Brown JA (1996) Does kin-biased territorial behavior increase kin-biased foraging in juvenile salmonids. Behav Ecol 7:24–29
Brown GE, Brown JA, Crosbie AM (1993) Phenotype matching in juvenile rainbow trout. Anim Behav 46:1223–1225
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. Ann Rev Ecolog Syst 18:287–368
Courtenay SC, Quinn TP, Dupuis HMC, Groot C, Larkin PA (1997) Factors affecting the recognition of population-specific odours by juvenile coho salmon. J Fish Biol 50:1042–1060
Dowling TE, Moore WS (1986) Absence of population subdivision in the common shiner *N. cornutus* (Cyprinidae). Environ Biol Fishes 15:151–155
Dugatkin LA, Wilson DS (1992) The prerequisites for strategic behaviour in bluegill sunfish *Lepomis macrochirus*. Anim Behav 44:223–230
Ferguson MM, Noakes DLG (1981) Social grouping and genetic variation in common shiners, *Notropis cornutus* (Pisces, Cyprinidae). Environ Biol Fishes 6:357–360
Fontaine PM, Dodson JJ (1999) An analysis of the distribution of juvenile Atlantic salmon (*Salmo salar*) in nature as a function of relatedness using microsatellites. Mol Ecol 8:189–198
Frommen J, Bakker TCM (2004) Adult threespine sticklebacks prefer to shoal with familiar kin. Behaviour 141:1401–1409
Gerlach G, Schardt U, Eckmann R, Meyer A (2001) Kin-structured subpopulations in Eurasian perch (*Perca fluviatilis* L.). Heredity 86:213–221
Griffiths SW, Armstrong JD (2001) The benefits of genetic diversity outweigh those of kin association in a territorial animal. Proc R Soc Lond B Biol Sci 268:1293–1296
Griffiths SW, Magurran AE (1999) Schooling decisions in guppies (*Poecilia reticulata*) are based on familiarity rather than kin recognition by phenotype matching. Behav Ecol Sociobiol 45:437–443
Hamilton WD (1964) The genetical evolution of social behaviour. J Theor Biol 7:1–16
Hasler AD, Scholz AT (1983) Olfactory imprinting and homing in salmon. Springer, Berlin Heidelberg New York
Hauser L, Carvalho GR, Pitcher TJ (1998) Genetic population structure in the Lake Tanganyika sardine *Limnothrissa miodon*. J Fish Biol 53(Suppl A):413–429
Helfman GS (1984) School fidelity in fishes: the yellow perch pattern. Anim Behav 32:663–672
Höglund LB (1961) The reaction of fish in concentration gradients. Rep Inst Freshw Res Drottningholm 43:1–147
Höjesjö J, Johnsson JI, Petersson E, Järvi T (1998) The importance of being familiar: individual recognition and social behavior in sea trout (*Salmo trutta*). Behav Ecol 9:445–451
Irwin DE, Bensch S, Price TD (2001) Speciation in a ring. Nature 409:333–337
Krause J, Butlin RK, Peuhkuri N, Pritchard VL (2000a) The social organization of fish shoals: a test of the predictive power of laboratory experiments for the field. Biol Rev Camb Philos Soc 75:477–501
Krause J, Hoare DJ, Croft D, Lawrence J, Ward A, Ruxton GD, Godin JG, James R (2000b) Fish shoal composition: mechanisms and constraints. Proc R Soc Lond B Biol Sci 267:2011–2017
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. Mol Ecol 9:993–1011
Lynch M (1991) The genetic interpretation of inbreeding depression and outbreeding depression. Evolution 45:622–629
Mann KD, Turnell ER, Atema J, Gerlach G (2003) Kin recognition in juvenile zebrafish (*Danio rerio*) based on olfactory cues. Biol Bull 205:224–225
Mathieu E, Roux AM, Bonhomme F (1990) Épreuves de validation dans l'analyse de structures génétiques multivariées: comment tester l'équilibre panmitique? Rev Stat Appl 38:47–66
Mayr E (1963) Animal species and evolution. Harvard University Press, Cambridge
Milinski M (1987) Tit-for tat in sticklebacks and the evolution of cooperation. Nature 325:433–437
Naish KA, Carvalho GR, Pitcher TJ (1993) The genetic structure and microdistribution of shoals of *Phoxinus phoxinus*, the European minnow. J Fish Biol 43(Suppl A):75–89
Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York
Olsén KH (1986) Chemoattraction between juveniles of two sympatric stocks of Arctic charr (*Salvelinus alpinus* (L.)) and their gene frequency of serum esterases. J Fish Biol 28:221–231
Olsén KH (1989) Sibling recognition in juvenile Arctic charr, *Salvelinus alpinus* (L.). J Fish Biol 34:571–581
Olsén KH (1992) Kin recognition in fish mediated by chemical cues. In: Hara TJ (ed) Fish chemoreception. Chapman & Hall, London, pp 229–248
Olsén KH, Grahn M, Lohm J, Langefors A (1998) MHC and kin discrimination in juvenile Arctic charr, *Salvelinus alpinus* (L.). Anim Behav 56:319–327
Olsén KH, Grahn M, Lohm J (2002) Influence of MHC on sibling discrimination in Arctic charr, *Salvelinus alpinus* (L.). J Chem Ecol 28:21–40
Penn DJ, Potts WK (1999) The evolution of mating preferences and major histocompatibility complex genes. Am Nat 153:145–164
Peuhkuri N, Seppae P (1998) Do three-spined sticklebacks group with kin? Ann Zool Fenn 35:21–27
Pouyaud L, Desmarais E, Chenuil A, Agnese JF, Bonhomme F (1999) Kin cohesiveness and possible inbreeding in the mouthbrooding tilapia *Sarotherodon melanotheron* (Pisces Cichlidae). Mol Ecol 8:803–812
Quinn TP, Busack CA (1985) Chemosensory recognition of siblings in juvenile coho salmon (*Oncorhynchus kisutch*). Anim Behav 33:51–56
Quinn TP, Hara TJ (1986) Sibling recognition and olfactory sensitivity in juvenile coho salmon. Can J Zool 64:921–925
Reusch TBH, Häberli MA, Aeschlimann PB, Milinski M (2001) Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. Nature 414:300–302
Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York
Shaw KL, Herlihy DP (2000) Acoustic preference functions and song variability in the Hawaiian cricket *Laupala cerasina*. Proc R Soc Lond B Biol Sci 267:577–584
Sherman PW, Reeve HK, Pfennig DW (1997) Recognition systems. In: Krebs JR, Davies NB (eds) Behavioral ecology: an evolutionary approach. Blackwell, Oxford, pp 69–96
Shields WM (1982) Philopatry, inbreeding and the evolution of sex. State University of New York Press, Albany

Stabell OB (1982) Detection of natural odorants by Atlantic salmon parr using positive rheotaxis olfactometry. In: Brannon EL, Salo EO (eds) Proceedings of the salmon and trout migratory behavior symposium. University of Washington Press, Seattle, pp 71–78

Stabell OB (1987) Intraspecific pheromone discrimination and substrate marking by Atlantic salmon parr. J Chem Ecol 13:1625–1643

Steck N, Wedekind C, Milinski M (1999) No sibling odour preference in juvenile threespined sticklebacks. Behav Ecol 10:493–497

Tang-Martinez Z (2001) The mechanisms of kin discrimination and the evolution of kin recognition in vertebrates: a critical re-evaluation. Behav Processes 53:21–40

Van Havre N, Fitzgerald GJ (1988) Shoaling and kin recognition in the threespine stickleback (*Gasterosteus aculeatus* L.). Biol Behav 13:190–201

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. Ecol Freshw 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. Anim Behav 52:371–378

Ward AJ, Krause J (2004) The effects of habitat- and diet-based cues on association preferences in three-spined sticklebacks. Behav Ecol 15:925–929