

SHORT COMMUNICATION

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

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Abstract

Previous microsatellite analysis showed that two subpopulations of perch (*Perca fluviatilis* L.) exist in Lake Constance. This raises questions of whether (i) Lake Constance was colonized by two populations that diverged in allopatry, or (ii) the two subpopulations 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 10 haplotypes. We suggest colonization via the Danube river, based on the frequency and dispersion of haplotypes, and knowledge of the lake's palaeohydrological development. Pairwise F_{ST} -values using mitochondrial DNA sequences showed no significant population subdivision. Our study provides strong evidence that subpopulations of perch in Lake Constance have diverged in sympatry.

Keywords: control region, Europe, postglacial colonization, mtDNA, *Perca fluviatilis* L., sympatric differentiation

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Introduction

Pleistocene glaciations not only widely formed today's landscapes, but also had major influences on patterns of animal dispersal and colonization (Avice 1992; Hewitt 1996, 2001; Bernatchez & Wilson 1998; Taberlet *et al.* 1998). In Europe, wide areas of Scandinavia and the Alps were repeatedly covered by ice sheets during the Pleistocene glaciations. Glacial advances and retreats formed lakes and reorganized river channels and drainages. During the Pleistocene glaciations the Rhine glacier coming from the inner Alps excavated the deep pre-alpine Lake Constance. The earliest colonization of Lake Constance by fish may 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; Nesbø *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, and the second most common species in Lake Constance. 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 found in the Upper Lake (population 1), which is deep, oligotrophic and warm-monomictic. The other population is found mainly 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 phylogeographical 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 postglacially; and (ii) the two subpopulations reflect genetic differences that have developed in sympatry after the postglacial colonization.

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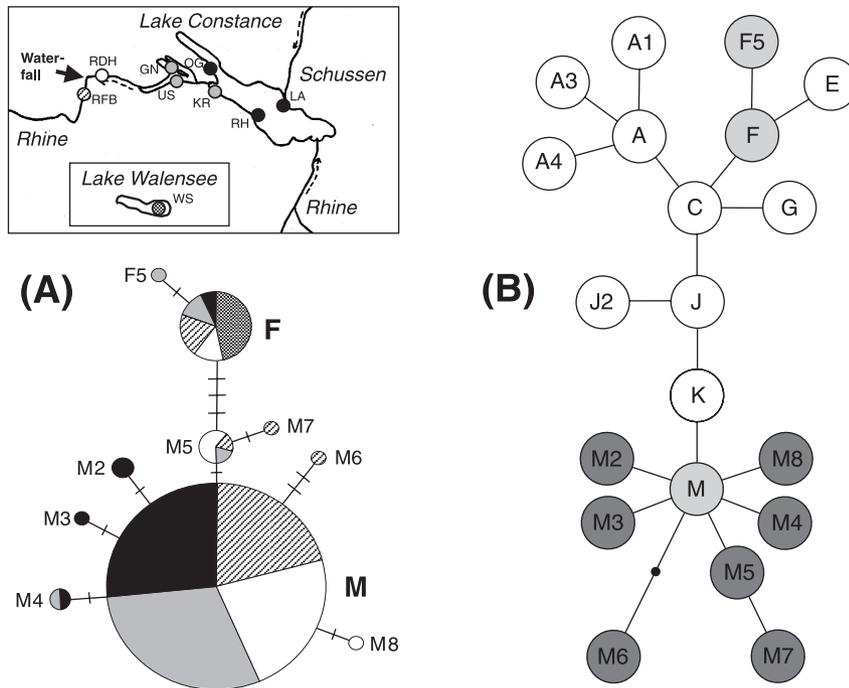


Fig. 1 Haplotype networks from mtDNA sequence data of Eurasian perch generated by statistical parsimony as implemented by *tcs* (v. 1.13; Clement *et al.* 2000). Haplotype designations refer to Nesbø *et al.* (1999) and this study. (A) Haplotype network for populations from Lake Constance, the two Rhenian localities and Lake Walensee. Black lines connect haplotypes, short bars reflect the number of mutational steps between haplotypes. Circle sizes represent haplotype frequencies, and fill patterns refer to geographical 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 grey circles represent haplotypes found in this study, white circles represent haplotypes found by Nesbø *et al.* (1999). Light grey circles represent haplotypes found in both studies, small black circles represent missing haplotypes.

Materials and methods

Sampling, DNA extraction, amplification and sequencing

Eighty-two perch were analysed 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 three and seven new perch were caught. Additional perch were caught in the River Rhine, 17 upstream and 18 downstream of the waterfall (Fig. 1A). Muscle tissue was stored in 80% ethanol until DNA extraction. Genomic DNA was extracted according to standard salt extraction procedures (Sambrook *et al.* 1989). To amplify 365 bp of the mtDNA D-loop the primers HV2 and CSB-D (Nesbø *et al.* 1998a) were used under the published reaction conditions. Polymerase chain reaction (PCR) products were purified using the GFX PCR/DNA and Gel Band Purifications-kit (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.

Data analysis

Alignment of mtDNA sequences was done by eye and a haplotype network was calculated using the computer program *tcs* (v. 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.

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

Results

We found 10 different haplotypes based on 11 variable sites among Eurasian perch sampled at 9 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. 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 five individuals). Haplotypes M2–M8 seem to be unique to Lake Constance and the two nearby Rhenian localities and to date have not been found elsewhere in the European drainages sampled (Nesbø *et al.* 1998b, 1999; Refseth *et al.* 1998).

A haplotype network (Fig. 1A) demonstrates the relationships between the different haplotypes. The two main haplotypes M and F are separated by four mutational 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. 1B).

Mitochondrial DNA 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 1). 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 1).

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 originate from colonization by two different mtDNA lineages, because the split between them is much older than the last glaciation (see Nesbø *et al.* 1999). In 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 drawn 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 led 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 behavioural 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 because nearly all the derived haplotypes

Table 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

	Population 1	Population 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).

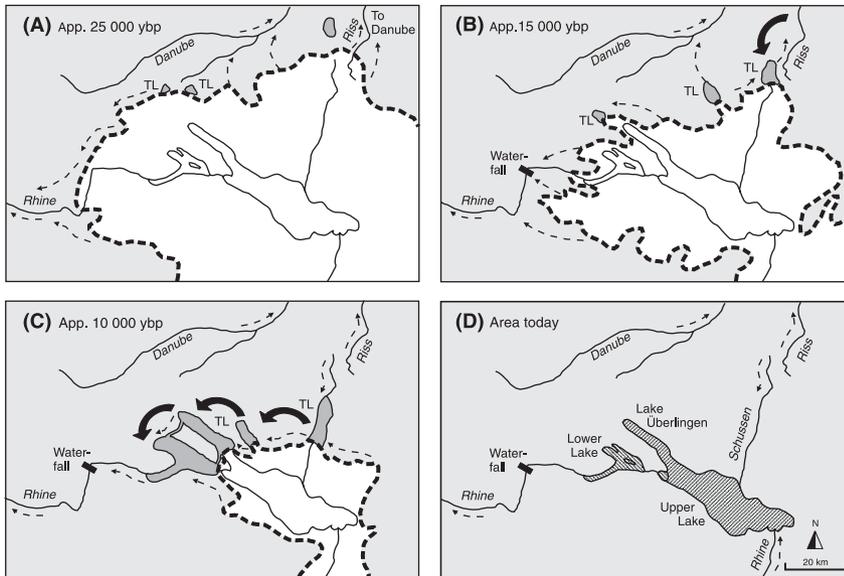


Fig. 2 Map of the drainage area of Lake Constance, simplified from Keller & Krays (2000) during and after the last (Würm) glaciation. Dashed arrows indicate directions of water flow. (A–C) Withdrawal of the glacier during three recessional stages. White area, glacier; TL, temporal proglacial lake; black 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.

(M2, M3, M6, M7 and M8) were found at only individual localities, whereas the ancestral M-haplotype was found at all localities. However, the question remains, whether the haplotypes derived from M (and F in one case) could have evolved during the very short time span of ~ 15 000 years. Divergence rates of 6–7% for the 5'-end of the D-loop have been suggested for some perciform species such as 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 was mainly found in the western part of the Danube (exceptions: one individual each in the rivers Tiber and Prut; Nesbø *et al.* 1999), where it co-occurs with the F-haplotype in a proportion of ~ 50% each haplotype. Within Lake Constance, 75% of all individuals were of the M-haplotype whereas only three individuals (7.5%) belonged to the F-haplotype (the remaining 17.5% 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 may 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 dis-

advantage 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. 1) with seven derived haplotypes would seem to imply extreme demographic expansion and dominance, whereas only one derived F-haplotype was found.

By contrast, 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). This natural barrier probably precluded perch from the Rhine drainage colonizing Lake Constance. Even a highly migratory fish species such as the Atlantic salmon (*Salmo salar* L.) was not able to overcome the waterfall and did not 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 a glacier (Fig. 2A–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 & Krays 2000). These temporal lakes could have been used by perch to colonize Lake Constance. Figure 2(A–C) shows a likely scenario of colonization by M-haplotypes from the Danube via one of these temporal lakes. As the glacier retreated, a drainage to the River Rhine became ice-free. From then on melt water 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 glacier withdrawal (Fig. 2C).

One question, however, remains, why did the M-haplotype not colonize more downstream regions 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 (see Appendix). 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). Very similar results were obtained for brown trout (*Salmo 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 have existed since the last glaciation which might 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 may have accumulated during their geographical isolation, which then limited introgressive hybridization between them. Furthermore, 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 may then have prevented the M-haplotype from colonizing the Rhine drainage system, a hypothesis that needs further testing.

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- This project is part of our research on population genetics, behaviour and competitive interactions with other species, of Eurasian perch in a large oligo- to mesotrophic prealpine lake. The study was carried out at the University of Constance as part of Jasminca Behrmann-Godel's PhD project. Gabriele Gerlach's research work focuses on population genetics to explain dispersal patterns and social structure in a variety of different species. Reiner Eckmann is a fish ecologist interested in the analysis of species interactions in littoral and pelagic habitats of large, oligotrophic lakes.
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Appendix

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	<i>N</i>	Haplotypes (<i>n</i>)
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 five loci (Gerlach *et al.* 2001).

†mtDNA data from Nesbø *et al.* (1999).

‡(dw), downstream of the waterfall; (uw), upstream of the waterfall.