Phylogeography of the barbel (*Barbus barbus*, Linnaeus 1758) in the area of Lake Constance

By Marina Lehmann

2006

Supervisor: Dr. Walter Salzburger

Bachelor Thesis

Department of Biology

Lehrstuhl für Zoologie & Evolutionsbiologie

University of Konstanz

Konstanzer Online-Publikations-System (KOPS)
URL: http://nbn-resolving.de/urn:nbn:de:bsz:352-0-253010

Index of Contents

1 Introduction

1.1 Phylogeography

Phylogeography is a rather young discipline. Its definition was introduced by Avise et al. in 1987 to describe obvious correlations between phylogenetics and historical biogeography, which emerged after the growing use of DNA sequences especially of mitochondrial DNA. Since then the number of publications using phylogeography as a title or as a keyword increased, which is representative for a much larger number of studies dealing with it but not naming the issue. Phylogeographic approaches seek to explain the influence of historical events on genetic structure contrary to contemporary influences. These contemporary influences such as population size, demographic fluctuations, migration and selection lead to species-specific signals, whereas different species showing congruent genetic structure have to be linked by strong ecological associations or have to be influenced by the same geological processes (Gagnon & Angers 2006).

1.2 Glaciation in the study area

Glaciations are among the most prominent historical factors influencing the present distribution of species. In the study area, the region of the pre-alpine Lake Constance, the last and most important glaciation was the Würm glaciation. It took place about 115,000 to 10,000 years ago and covered the Alps and its forelands (Fig. 1).

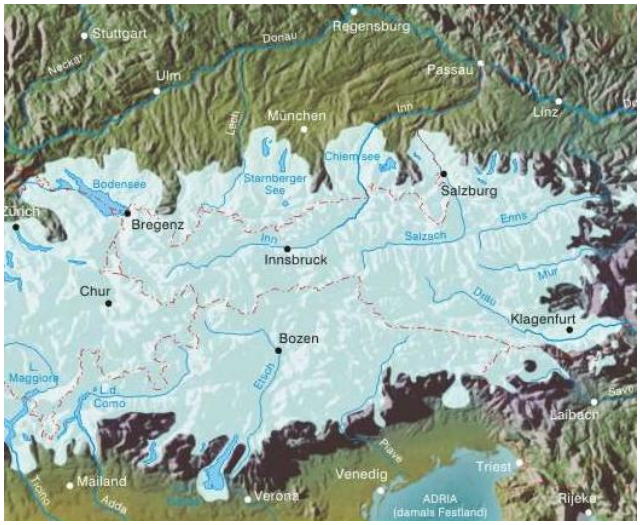


Fig. 1: Alpine Würm glaciation

As the glaciers retreated temporary lakes and rivers were formed, which connected the Rhine and the Danubian drainage systems (Fig. 2). Previous studies showed that fish species such as the vairone (*Leuciscus souffia*, Risso 1826) (Salzburger et al. 2003), the Eurasian perch (*Perca fluviatilis*, Linnaeus 1758) (J. Behrmann-Godel et al. 2004) and the chub (*Leuciscus cephalus*, Linnaeus 1758) (F. Muenzel unpublished) recolonized the area of Lake Constance from Danubian refuges through these temporary lakes and rivers.

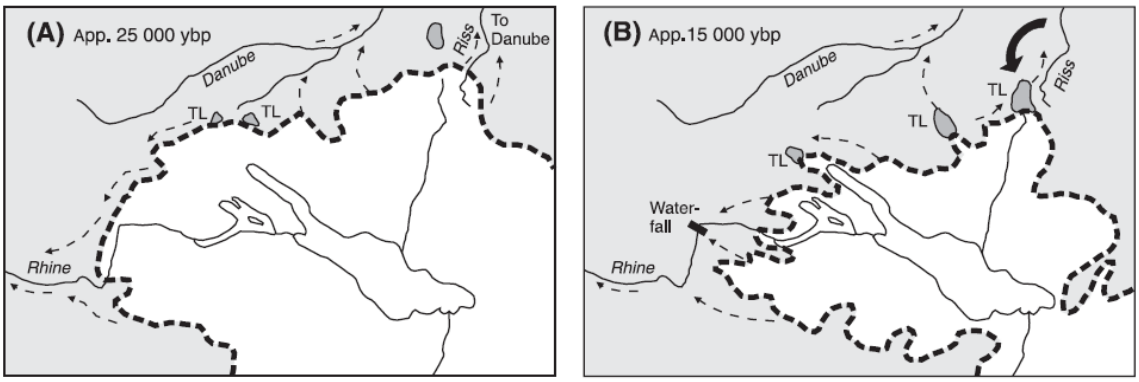


Fig. 2: Expansion of Rhine glacier

1.3 The barbel

The subfamily of the barbs belongs to the family Cyprinidae and covers almost half of the species of this family. The common barbel (*Barbus barbus*) grows up to 90 cm and has two pairs of barbels that led to its name. The barbel prefers the middle stretches of rather rapidly flowing rivers where it feeds mainly on insect larvae and small crustaceans, but also on fish spawn and carrion. In search for food and spawning grounds, common barbels move up to 10 km per day. Therefore, the common barbel is much more widespread than other European Barbus species. It is prevalent from France to the Black Sea but is absent in the Mediterranean, parts of the United Kingdom and Scandinavia replaced by *B. plebejus* in Italy and *B. meridionalis* from Spain to the South of France and Northern Italy to the Balkans. In fact there are over 30 different morphs treated as species or subspecies, depending on the author (Gerstmeier & Romig 1998). A previous study on the phylogeography of the barbel in Europe revealed a close relationship between the fishes from the Danube and Rhine system, but could not resolve the situation in detail (Kotlik & Perrebi 2001).

1.4 Hypothesis

The haplotype networks of the chub and the vairone show a quite corresponding pattern differing in relation to their adaptability on their environment. The vairone is a more specialised species and therefore restricted to narrow habitats. The chub in contrast is a generalist, being able to live in diverse environments. These traits become visible as different schemes in the haplotype network.

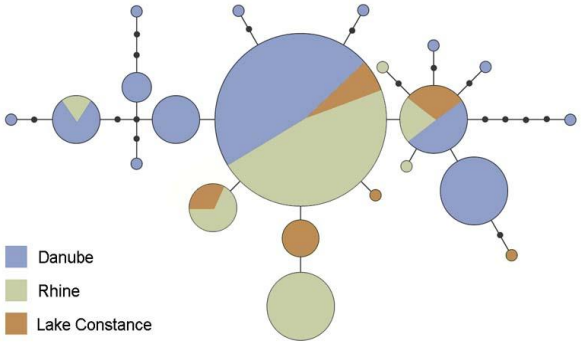


Fig. 3: Haplotype network of *L. cephalus* (F. Muenzel unpublished)

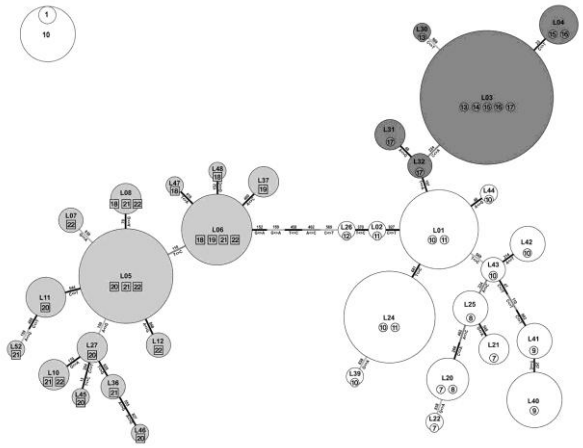


Fig. 4: Haplotype network of *L. souffia* (Salzburger et. al 2003)

Both networks show a more or less strong division into a Rhine and a Danubian cluster. All in all three haplotypes of *L. cephalus* appear in both drainage systems, which are not as distinctly separated (Fig. 3). For *L. souffia*, there is only one mutation clearly separating the groups (Fig. 4). Both networks display a more complex Danubian cluster as a result of the Danubian refuge function. We expected the barbel to fit into the pattern resulting from the fish species mentioned above. The Danubian haplotype group should show more variation being the older one and being the source for dispersal into the Rhine drainage after glaciation (see Fig. 2 (B)).

2 Materials and Methods

2.1 Mitochondrial control region (d-loop)

The mitochondrial control region is noncoding so that mutations accumulate to a higher rate as compared to protein-coding sections. It consists of a central conserved domain that is bounded by two highly variable domains. The control region itself is bordered by the genes cytochrome *b* and 12S rRNA (Fig. 5). There are several advantages for the use of mitochondrial DNA. First, it is inherited only via the maternal path, so recombination is avoided. Second, highly conserved regions allow comparisons on larger scales whereas highly variable regions allow to distinguish even between subspecies or local variants. Further, the three conserved regions permit the use of universal primers.

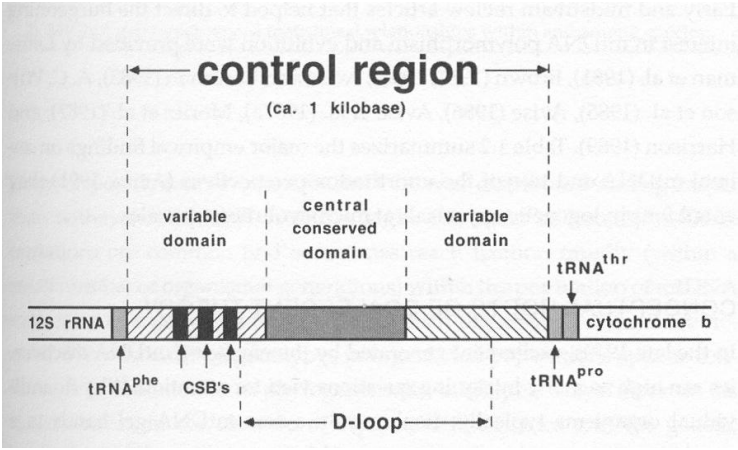


Fig. 5: Mitochondrial control region

2.2 Procedures

2.2.1 Sampling

Samples had been taken in 10 different rivers in Switzerland, Southern Germany and Austria giving an overall of 63 samples. Fin clips were taken and stored in 96 % ethanol. A detailed list of specimens, their sampling locality and sampling date are given in Table 1.

sample number	Name	sampling place	sampler	sampling date
ML01	B. barbus	Zollenreuter Aach	E. Hespeler	Sept.'05
ML02	B. barbus	Zollenreuter Aach	E. Hespeler	Sept.'05
ML03	B. barbus	Zollenreuter Aach	E. Hespeler	Sept.'05

ML04	B. barbus	Zollenreuter Aach	E. Hespeler	Sept.'05
ML05	B. barbus	Zollenreuter Aach	E. Hespeler	Sept.'05
ML06	B. barbus	Zollenreuter Aach	E. Hespeler	Sept.'05
ML07	B. barbus	Zollenreuter Aach	E. Hespeler	Sept.'05
ML08	B. barbus	Zollenreuter Aach	E. Hespeler	Sept.'05
ML09	B. barbus	Zollenreuter Aach	E. Hespeler	Sept.'05
ML10	B. barbus	Zollenreuter Aach	E. Hespeler	Sept.'05
ML11	B. barbus	Zollenreuter Aach	E. Hespeler	Sept.'05
ML12	B. barbus	Zollenreuter Aach	E. Hespeler	Sept.'05
ML13	B. barbus	Zollenreuter Aach	E. Hespeler	Sept.'05
ML14	B. barbus	Zollenreuter Aach	E. Hespeler	Sept.'05
ML15	B. barbus	Rhein/Schaffhausen	Egloff	/
ML16	B. barbus	Rhein/Schaffhausen	Egloff	/
ML17	B. barbus	Rhein/Schaffhausen	Egloff	/
ML18	B. barbus	Rhein/Schaffhausen	Egloff	/
ML19	B. barbus	Rhein/Schaffhausen	Egloff	/
ML20	B. barbus	Rhein/Schaffhausen	Egloff	/
ML21	B. barbus	Mangfall/Rosenheim	M. Sanetra/E. Hespeler	/
ML22	B. barbus	Mangfall/Rosenheim	M. Sanetra/E. Hespeler	/
ML23	B. barbus	Donau/Niederalteich	M. Sanetra/E. Hespeler	/
ML24	B. barbus	Donau/Niederalteich	M. Sanetra/E. Hespeler	/
ML25	B. barbus	Uffinger Aach/Heimgarten	M. Sanetra/E. Hespeler	/
ML26	B. barbus	Uffinger Aach/Heimgarten	M. Sanetra/E. Hespeler	/
ML27	B. barbus	Uffinger Aach/Heimgarten	M. Sanetra/E. Hespeler	/
ML28	B. barbus	Uffinger Aach/Heimgarten	M. Sanetra/E. Hespeler	/
ML29	B. barbus	Uffinger Aach/Heimgarten	M. Sanetra/E. Hespeler	/
ML30	B. barbus	Eger/Lierheim	M. Sanetra/E. Hespeler	/
ML31	B. barbus	Eger/Lierheim	M. Sanetra/E. Hespeler	/
ML32	B. barbus	Eger/Lierheim	M. Sanetra/E. Hespeler	/
ML33	B. barbus	Eger/Lierheim	M. Sanetra/E. Hespeler	/
ML34	B. barbus	Eger/Lierheim	M. Sanetra/E. Hespeler	/
ML35	B. barbus	Paar/Ottmaring	M. Sanetra/E. Hespeler	/
ML36	B. barbus	Paar/Ottmaring	M. Sanetra/E. Hespeler	/
ML37	B. barbus	Paar/Ottmaring	M. Sanetra/E. Hespeler	/
ML38	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML39	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML40	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML41	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML42	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML43	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML44	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML45	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML46	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML47	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML48	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005

ML49	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML50	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML51	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML52	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML53	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML54	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML55	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML56	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML57	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML58	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML59	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML60	B. barbus	Bregenzer Aach	M. Barluenga/E. Hespeler	24.05.2005
ML61	B. barbus	Saane	/	/
ML62	B. barbus	Saane	/	/
ML63	B. barbus	Inselhotel/Rhein Konstanz	/	/

Table 1: Sampling list

2.2.2 DNA highsalt extraction

A small piece of the fin clip was put into a reaction tube, where 310 µl extraction buffer, 80 µl 10% SDS and 10 µl 1% Proteinase K were added. The tube was incubated at 37°C over night and centrifuged for 5 min at 13200 rpm. The supernatant was transferred into a new reaction tube, while the pellet was discarded. 180 µl 5M NaCl were added and mixed by turning the tubes. Again the sample was centrifuged for 5 min at 13200 rpm and the supernatant was transferred into a new reaction tube. This time 420 µl cooled isopropanole were added to the supernatant, mixed by turning the reaction tube and centrifuged for 5 min at 13200 rpm. The supernatant was carefully pipetted off, leaving the DNA in the pellet. 250 µl of 70% ethanol were added to wash the pellet by turning the tube. The reaction tube was centrifuged for 5 min at 13200 rpm. Afterwards the supernatant was taken off carefully. This washing step was repeated. Finally the pellet was airdried and reeluted in 100 µl HPLC-water overnight.

2.2.3 PCR

First, the polymerase chain reaction was optimised. Different primer pairs were tested. The best one turned out to be L-Pro-F (5'- AAC TCT CAC CCC TAG CTC CCA AAG -3') and TDK-D (5'- CCT GAA GTA GGA ACC AGA TG -3'). For this pair a temperature gradient PCR was conducted under following conditions:

94°C	60 sec		
94°C	30 sec	}	40 x
46°C – 56°C	30 sec		
72°C	90 sec		
72°C	420 sec		

reaction mix:	
$H_2O_{dest.}$	15, 0 µl
10 mM dNTPs	2, 5 µl
10 x buffer	2, 5 µl
P1 (L-Pro-F)	1, 0 µl
P2 (TDK-D)	1, 0 µl
Taq	1, 0 µl
DNA	2, 0 µl
total	25, 0 µl

The optimal temperature was found to be 46°C.

2.2.4 Gel electrophoresis

To check if the PCR was successful, a 1, 5 % agarose gel was loaded with 5 µl of the PCR product (Figs. 6, 7). 35 ml gel were charged with 2, 5 – 3, 0 µl ethidiumbromide. The electrophoresis was run for 35 min at 90 V.

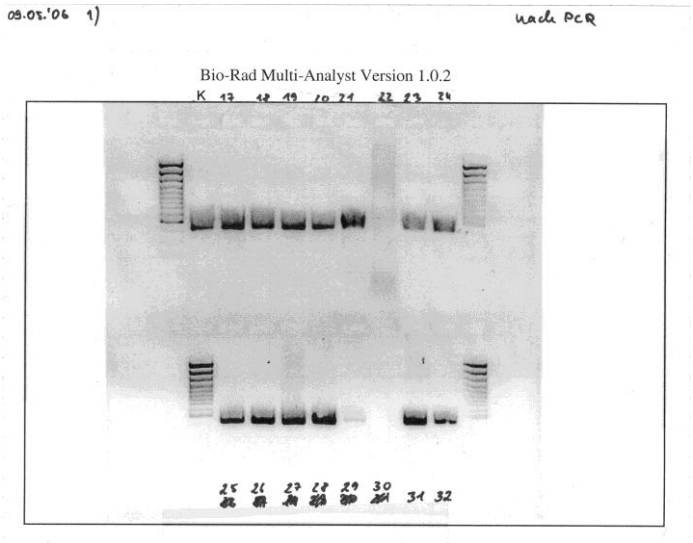


Fig. 6: Example of an agarose gel after PCR

2.2.5 Purification

For purification the QIAquick PCR Purification Kit (Qiagen) was used. 100 µl PB buffer were added to the sample, mixed by vortexing and applied on a QIAquick column. The column was then centrifuged for 60 sec at 13200 rpm. Flow-through was discarded, 750 µl of 35% guanidine hydrochloride solution were added and centrifuged under same conditions. 750 µl of PE buffer were applied on the column and centrifuged. This step was repeated and the sample as centrifuged another 60 seconds. Next, the column was placed in a new reaction tube. 50 µl EB buffer were added, the column was left standing for 60 sec and then centrifuged for 60 sec at 13200 rpm. The purified DNA was stored at -20 °C.

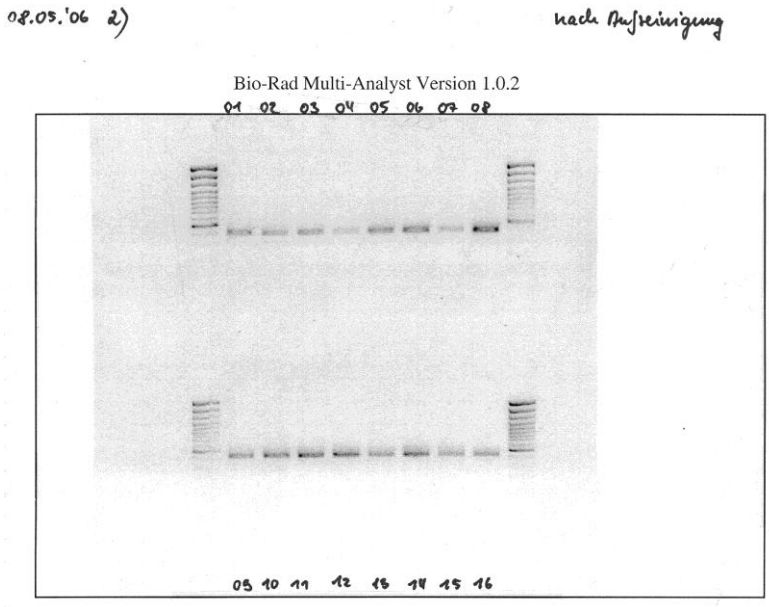


Fig. 7: Example of an agarose gel after purification

2.2.6 Cycle sequencing

With the purified DNA a cycling PCR was conducted under following conditions:

96°C	30 sec	
96°C	10 sec	35 x
50°C	20 sec	
60°C	240 sec	
4°C	420 sec	

Reaction mix:	
$H_2O_{dest.}$	4, 5 µl
TRM	1, 5 µl
Primer (forward or reverse)	1, 0 µl
NP40	1, 0 µl
DNA	2, 0 µl
Total	10, 0 µl

2.2.7 DNA precipitation

After the cycle sequencing PCR the samples had to be purified again. Precipitation was done by adding 1 µl NaOAc (pH 4, 6) and 25 µl 95 % ethanol to the sample, vortexing and centrifuging for 30 min at 13200 rpm. Supernatant was taken off carefully, 200 µl 70% ethanol were added, the reaction tube was vortexed and centrifuged for 5 min at 13200 rpm. This step was repeated. The samples were dried at RT, dissolved in 15 µl formamide and vortexed after 30 min.

2.2.8 Sequencing

The samples were loaded on a 96 well plate and sequenced with an ABI PRISM 3100 DNA sequencer (Applied Biosystems). The gained sequences were checked and revised with the Sequencher software (Genecodes). Forward and reverse sequences of ML01-ML07, ML09-ML15, ML17-ML21 and ML23-32 were automatically assembled by the software. The other sequences were sequenced either forward or reverse. Nucleotides, which were not recognised by the sequencing software, were inserted according to the electropherogram (see Fig. 8).

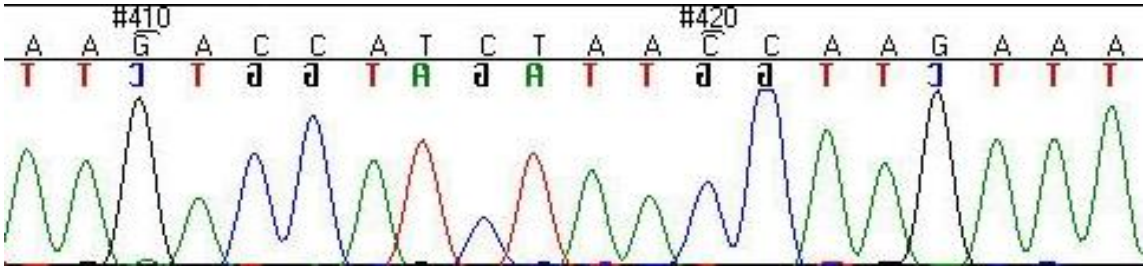


Fig. 8: Example of an electropherogram

2.3 Phylogeographic Analyses

2.3.1 Modeltest 3.06

Modeltest is a program for selecting the model of nucleotide substitution that best fits the given data out of 56 models, and implements three different model selection frameworks: hierarchical likelihood ratio tests (hLRTs), Akaike information criterion (AIC), and Bayesian information criterion (BIC). The program compares models with variable complexity based on a chi-square test. It was developed by Posada & Crandall 1998.

2.3.2 PAUP

PAUP means Phylogenetic Analysis Using Parsimony. It uses the results of Modeltest or works with the given data to create trees. This can be done with Neighbourhood Joining, Parsimony or Maximum Likelihood. Another feature is the creation of Absolute Distance Matrices. The used version was PAUP*4.0b10 (Swofford 2002).

2.3.3 ARLEQUIN (mismatch analysis)

Arlequin is a population genetics software, which includes a large amount of population genetics methods and statistical tests. These methods can be executed either at the intra-population or at the inter-population level. Different analyses can be chosen via a graphical interface which also allows to change options and parameters. Data can be imported in different formats such as DNA sequences, RFLP data, microsatellite data, standard data or allele frequency data. Additionally the program recognises different file formats. It was developed by Excoffier et al. 2005.

3 Results

3.1 Sequences

The 59 obtained sequences were aligned by hand with PAUP and cut to 389 bp length at both strand ends. This was possible as mutations only occurred in the central region (Fig. 9).

```
HT04  ML33F__EGL  CTTGAATAAACAACTAATATTGCATCGAAACATATTAATGTAGTAAGAGA
HT09  ML38F__AUS  CTTGAATAAACAACTAATATTGCATCGAAGTATATTAATGTAGTAAGAGA
HT06  ML61R__SAA  CTTGAATAAACAACTAATATTGCATCGAAGTATATTAATGTAGTAAGAGA
```

Fig. 9: Sequence examples of three different haplotypes from position 251 to 300 including two variable positions at 279 and 280

3.2 Phylogenetic tree

With these 59 sequences a first maximum parsimony tree was created with PAUP* (Fig. 10). This tree was unrooted. Of the 389 equally weighted characters, eight characters were parsimony-uninformative and 7 were parsimony-informative. Gaps were treated as “missing”.

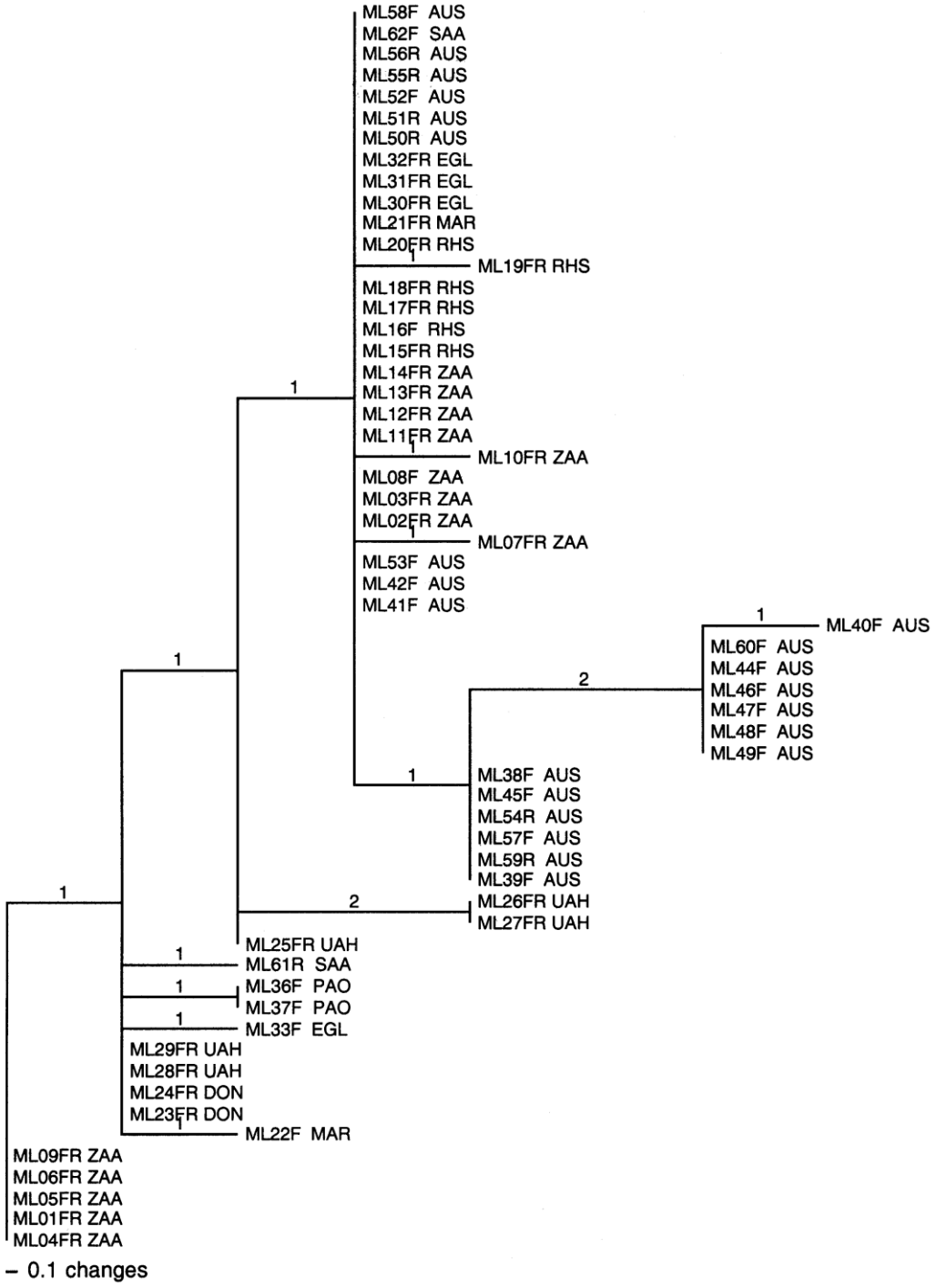


Fig. 10: Maximum parsimony tree

3.3 Haplotypes

Identical sequences were grouped to reduce datasets. Each group was represented by its most complete sequence (Table 2). This led to eleven different haplotypes resulting from ten variable positions (Table 3). One mutation occurred twice at position 279, but as can be seen later in the haplotype network independently. Therefore the consistency index (CI) was 0.500 for this mutation. The CI for all the other mutations was 1.000.

haplotype	sequence
HT01	ML01
HT02	ML22
HT03	ML23
HT04	ML33
HT05	ML36
HT06	ML61
HT07	ML25
HT08	ML26
HT09	ML38
HT10	ML19
HT11	ML32

Table 2: Sequences representing haplotypes

position	consistency index	nucleotide change
36	1.000	G => A
85	1.000	G => A
121	1.000	T => C
148	1.000	A => G
189	1.000	T => C
204	1.000	C => A
279	0.500	A => G
280	1.000	T => C
351	1.000	C => T
370	1.000	A => G

Table 3: Variable positions in the sequence alignment

For the reduced dataset again a parsimony tree was created with the same settings. This time seven variable characters were parsimony-uninformative, three were parsimony-informative. To verify this result, Modeltest software was used to select the best of 56 different models. The software determined Trn+I as best fitting model according to Akaike Information Criterion (AIC). The Log Likelihood Score was 574.2525, the AIC was 1158.5050. The parameters given by the model were implemented into PAUP* and led to a maximum likelihood tree (Fig. 11) looking exactly the same as the previously created parsimony tree.

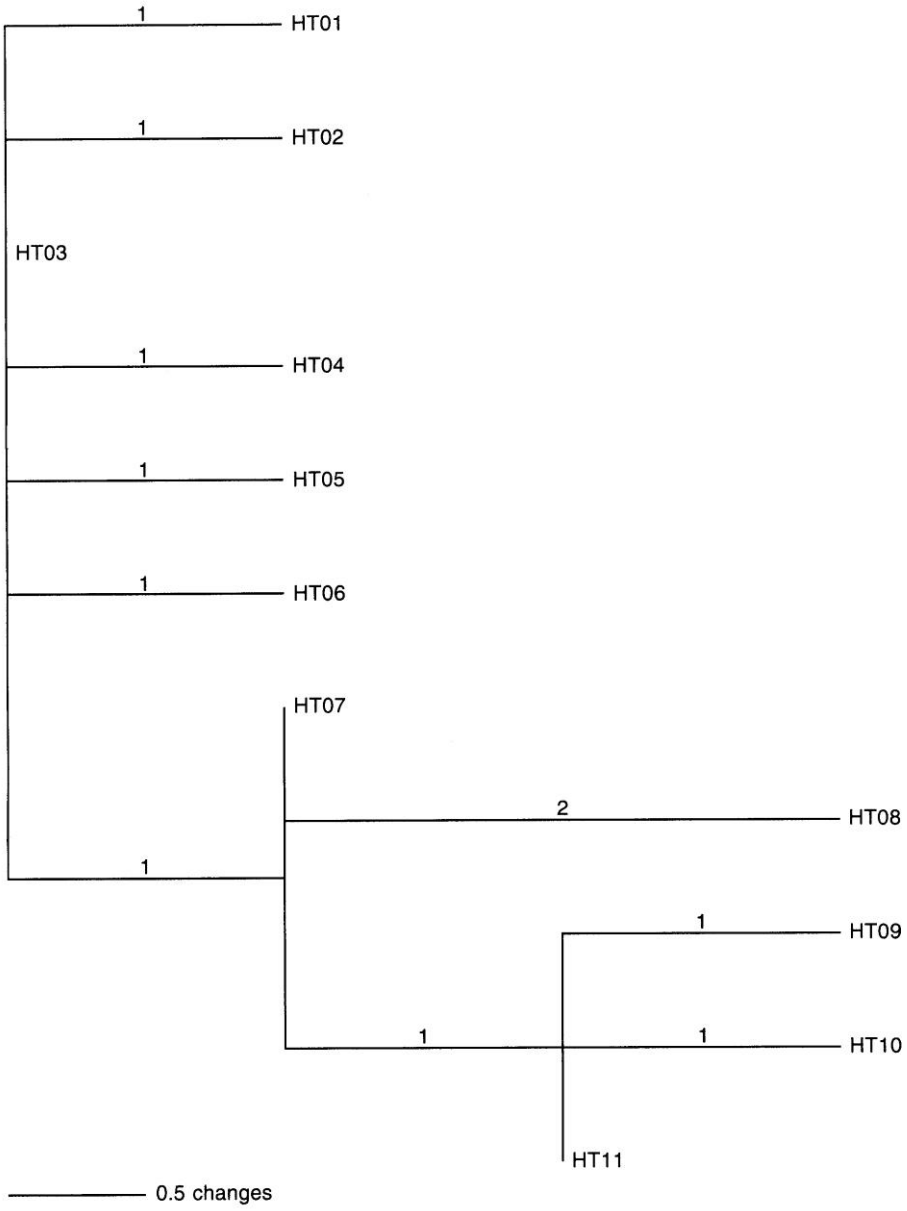


Fig. 11: Maximum likelihood tree

3.4 Haplotype network

The tree was transferred into a haplotype network (Fig. 12) by hand. Each circle represents one haplotype; the black dot stands for a non-existing haplotype. Each line represents one mutation. The circle area is proportional to the number of individuals, which belong to this haplotype. Different colours correspond to the different sampling localities (Fig. 13). Sampling places coloured in red and yellow belong to the Rhine drainage system, the ones in blue and green belong to the Danubian drainage system. The largest distance between two sampling places is about 500 km.

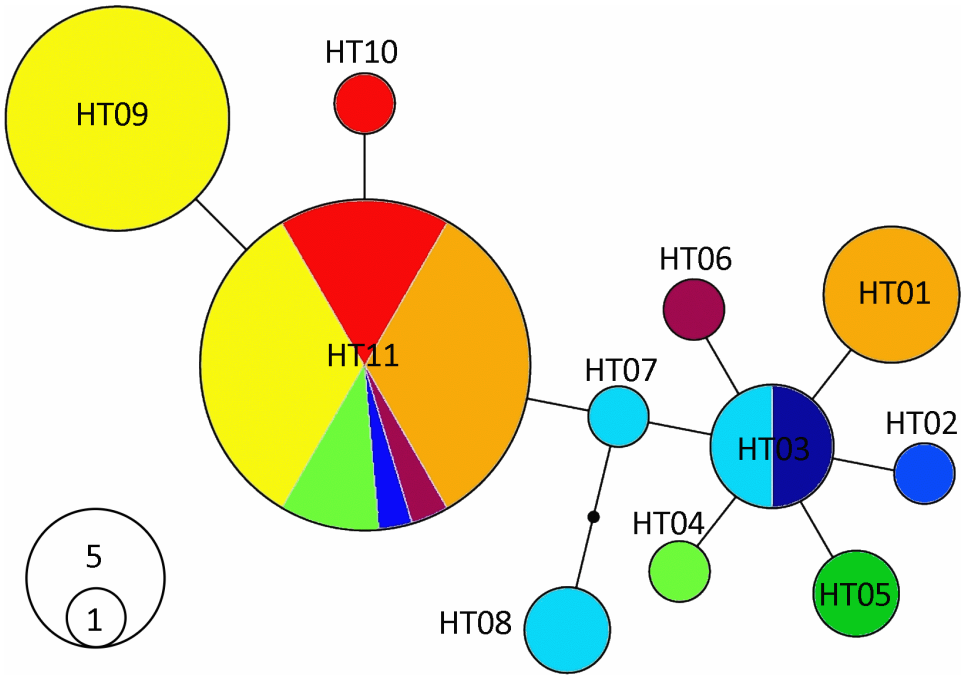


Fig. 12: Haplotype network. Colors refer to the sampling localities (see Fig. 13)

This network shows a division into two clusters. On one hand, haplotypes 09, 10 and 11, on the other hand the remaining eight haplotypes. This corresponds roughly with the geographic distribution of the sampling places. There is only one haplotype (HT05), which occurs only at one sampling place. Haplotype 11 is found in six different sampling places, even at those places, which are separated by almost 400 kilometres. There is no sampling place including haplotypes, which are separated by more than three mutations.

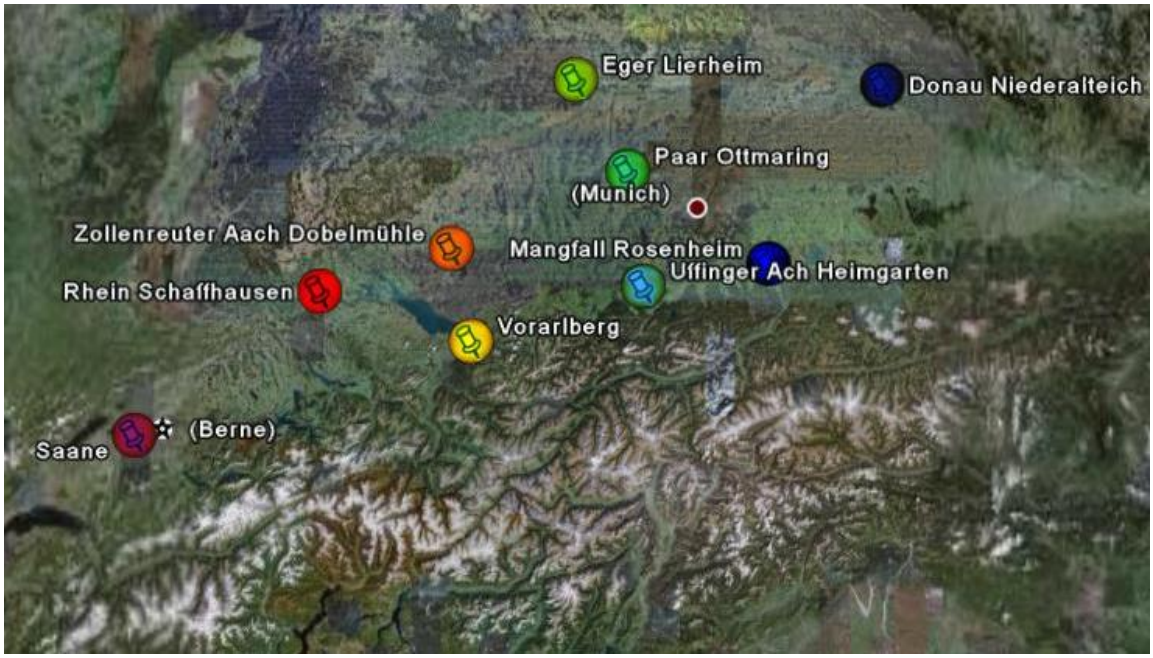


Fig. 13: Sampling places

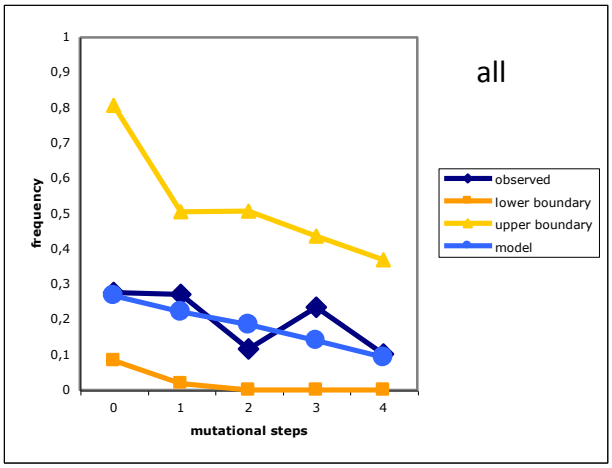
3.5 Mismatch analysis

A mismatch analysis was performed to show the demographic expansion of all haplotypes and of the Rhine haplotypes (Fig. 14, Table 4). It shows the distribution of the observed number of differences between pairs of haplotypes. Populations that have passed trough a recent demographic expansion are likely to be unimodal.

	τ	**observed mean**	θ_0	θ_1
All	3.205	1.975	0.409	362.639
Rhine	0.656	0.703	0.129	1566.047

Table 4: Results of mismatch analyses: expansion parameter (τ), mismatch observed mean, mutation parameter before expansion (θ_0) and mutation parameter after expansion (θ_1).

The data are based on a 99% confidence interval and 1000 replicates. The mismatch distribution of all haplotypes shows two peaks, which means that there were two different expansions. If only Rhine haplotypes were compared, the distribution was unimodal with a maximum of two differences. This indicates a more recent expansion.



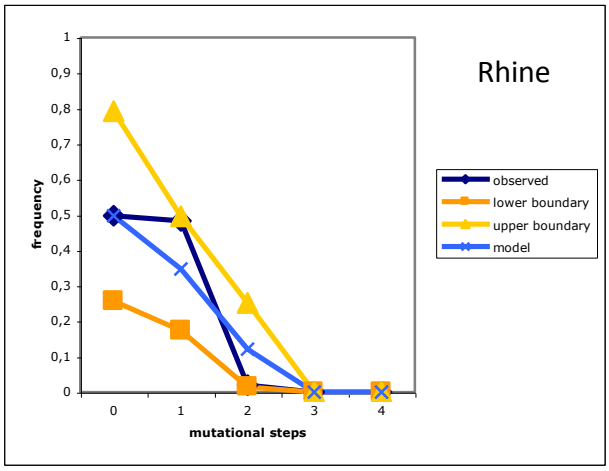


Fig. 14: Results of mismatch analysis

4 Discussion

Freshwater fish are good phylogeographic model systems, because they are restricted to their river drainages (Bernatchez & Wilson 1998). There have already been a series of phylogeographic studies with freshwater fish in Europe e.g. the vairone (*Leuciscus souffia*, Risso 1826) (Salzburger et al. 2003), Eurasian perch (*Perca fluviatilis*, Linnaeus 1758) (J. Behrmann-Godel et al. 2004) or the chub (*Leuciscus cephalus*, Linnaeus 1758) (F. Muenzel unpublished). A lot of these studies focussed on a larger geographic scale, covering the whole continent (Kotlik & Berrebi 2001, Salzburger et al. 2003). Therefore little is known about local patterns. Under this condition Lake Constance and its surroundings is a good model, including the two drainage systems of Rhine and Danube within a rather small-sized area and the previous glaciation forming the lake and the region. The barbel displays a relatively clear pattern (Figs. 12,13) with a distinct separation between Danube and Rhine. In the Danubian samples more haplotypes were found than in the Rhine samples, also with longer genetic distances between the haplotypes. This suggests that the Danubian population is older. The result is supported by the mismatch analysis (Fig. 14, Table 4), which shows two peaks for all samples meaning two different expansions. Rhine samples show an expansion only two mutations in the past, therefore they are younger in relation to the other samples.

There is one haplotype (HT06) that does not fit into the separation between Rhine and Danube. This sample was taken at the Saane River, belonging to the Rhine system, whereas the haplotype is grouped together with the Danubian ones. This

could be explained by human translocation, as the barbel is of some economic importance. Alternatively, this could be the result of a colonization of the Rhine system through a second lineage originating from the Danube drainage. As only two samples were taken at the Saane River, more data would be necessary to resolve this question. In the 14 samples taken at the Zollenreuter Aach, two very distinct haplotypes were found, HT11 and HT01. While HT11 is common in Rhine and Danube, HT01 is grouped together with Danubian haplotypes. This seems to be a consequence of the geographic intermediate position of the Zollenreuter Aach between Rhine and Danube.

In terms of haplotype number and genetic diversity, the barbel resembles the cases of *Leuciscus souffia* and *Leuciscus cephalus*. However, with respect to the colonisation of the area, the barbel occupies an intermediate position. In *L. cephalus* (Fig. 3), several lineages of Danubian origin seeded the Rhine system. In *L. souffia* (Fig. 4), the Rhine haplotypes are monophyletic. In the barbel at least one lineage seeded the Rhine system, although shared haplotypes are common (see e.g. HT11). This seems to reflect the ecologic conditions (Gagnon & Angers 2006). The stone loach is an example, that also other scenarios are possible (Barluenga & Meyer 2005). This species has colonised the area long before these last glaciations.

This study has shown that the analysis of the mitochondrial control region is a reliable method for phylogeography on small scales and has confirmed the existing colonisation scenario.

5 Zusammenfassung

Die Phylogeographie ist ein eher junges Feld. Sie wurde zum erstenmal von Avise et al. 1987 erwähnt, um offensichtliche Zusammenhänge zwischen Phylogenetik und historischer Biogeographie zu erklären. Seit dieser Zeit stieg die Zahl der Publikationen, welche den Begriff Phylogeographie im Titel führen oder als Schlagwort verwenden, stetig an. Faktoren wie Populationsgröße, Migration und Selektion führen zu artspezifischen Signalen in der genetischen Struktur. Wenn aber verschiedene Arten eine ähnliche genetische Struktur aufweisen, so weist dies auf gemeinsame Einflüsse durch historische und geologische Ereignisse hin (Gagnon & Angers 2006).

Eiszeiten gehören zu den wichtigsten Ereignissen, welche die heutige Verteilung von Arten beeinflusst haben. Die Würmeiszeit war die jüngste und wichtigste Eiszeit in der untersuchten Region um den Bodensee. Sie fand vor 115,000 bis 10,000 Jahren statt und bedeckte die ganze untersuchte Region. Während der Gletscherschmelze entstanden zeitlich begrenzte Seen und Flüsse, welche die Einzugsgebiete von Rhein und Donau verbanden. Vorherige Studien haben bereits gezeigt, daß Fische wie der Strömer (*Leuciscus souffia*, Risso 1826) (Salzburger et al. 2003), der Flussbarsch (*Perca fluviatilis*, Linnaeus 1758) (J. Behrmann-Godel et al. 2004) oder der Döbel (*Leuciscus cephalus*, Linnaeus 1758) (F. Muenzel unpublished) diese zeitweiligen Verbindungen nutzten, um die Gegend um den Bodensee über die Rückzugsgebiete aus der Donau wiederzubesiedeln.

Die Haplotypnetzwerke von Strömer und Döbel zeigen grosse Übereinstimmung, welche nur durch deren verschiedene Anpassungsfähigkeit an ihre Umwelt variieren. Es wurde erwartet, daß die Barbe ebenfalls diesem Schema entspricht.

Um dies zu bestätigen, wurden insgesamt 63 Proben aus dem süddeutschen Raum, Österreich und der Schweiz untersucht. Dies führte zu 59 Sequenzen mit einer Länge von 389 bp aus der mitochondriellen Kontrollregion, welche sich durch ihre hohe Variabilität besonders für innerartliche Vergleiche eignet. Der Vergleich ergab elf verschiedene Haplotypen, wobei mehr Haplotypen in der Donau-Population gefunden wurden. Das aus den Haplotypen erstellte Netzwerk zeigte deutliche Übereinstimmungen mit den Besiedlungs-Szenarien von Strömer (*L. souffia*) und Döbel (*L. cephalus*). Eine „mismatch analysis“ wies dazu auf die Trennung zwischen den Populationen aus den Einzugsgebieten von Rhein und Donau hin.

Diese Arbeit zeigt, dass die Analyse der mitochondrialen Kontrollregion auch auf lokaler Ebene gute Ergebnisse liefern kann.

6 Literature

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb Ca, Saunders NC (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics,* **18,** 459-522.

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.

Bernatchez L, Wilson CW (1998) Comparative phylogeography of Nearctic and Palearctic fishes. *Molecular Ecology*, **1**, 431-452.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47-50.

Gerstmeier R, Romig T (1998) Die Süßwasserfische Europas, pp. 275-280. Frankh-Kosmos Verlags-GmbH & Co., Stuttgart.

Gagnon M-C, Angers B (2006) The determinant role of temporary proglacial drainages on the genetic structure of fishes. *Molecular Ecology*, **15**, 1051-1065.

Kotlik P, Berrebi P (2001) Phylogeography of the barbel (Barbua barbus) assessed by mitochondrial DNA variation. *Molecular Ecology*, **10**, 2177-2185.

Muenzel, F. The phylogeography of L. souffia and L. cephalus in the area of Lake Constance (unpublished)

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, **14**, 817-818.

Salzburger W, Brandstätter A, Gilles A, Parson W, Hempel M, Sturmbauer C, Meyer A (2003) Phylogeography of the vairone (*Leuciscus souffia*, Risso 1826) in Central Europe. *Molecular Ecology*, **12**, 2371-2386.

Swofford DL (2002) PAUP*: Phylogentic Analysis Using Parsimony (and Other Methods). Sinauer Associates, Sunderland

7 Abbreviations

bp	basepairs
CI	consistency index
dNTPs	desoxyribonukleosidtriphosphates
HPLC	high performance liquid chromatography
µl	microlitres
min	minutes
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
rpm	rotations per minute
rRNA	ribosomal RNA
RT	room temperature
SDS	sodium dodecyl sulphate
sec	seconds

8 Danksagung

Hiermit möchte ich allen Danksagen, die mich während meines Studiums und der Erstellung dieser Bachelorarbeit unterstützt haben.

Meinen Eltern, die mir dieses Studium ermöglicht haben.

Elke Hespeler, die mich immer wieder mit all dem versorgt hat, was ich während meiner Zeit im Labor verbraucht habe.

Und natürlich Walter Salzburger, der mich während dieses Projektes betreut hat.

9 Erklärung

Hiermit erkläre ich, daß ich die vorliegende Arbeit selbständig verfaßt und keine anderen als die angegebenen Hilfsmittel verwendet habe. Die Stellen, die anderen Werken dem Wortlaut oder dem Sinn nach entnommen sind, habe ich durch Angabe der Quellen kenntlich gemacht.

Konstanz, den 10. Juli, 2006

Marina Lehmann