Populations genetics of freshwater pearl mussel (*Margaritifera Margaritifera*) and the host fish brown trout (*Salmo trutta*) in Rivers of Brittany and Normandy (France)

(Report 30 June 2014)

Jürgen Geist

Aquatic Systems Biology Unit, Department of Ecology and Ecosystem Management, Technische Universität München, Mühlenweg 22, 85350 Freising, Germany. E-mail: Geist@wzw.tum.de; Tel: ++498161713767; Fax: ++498161713477

LIFE09NATFR00583

Introduction

The freshwater pearl mussel (*Margaritifera margaritifera*) is one of the most critically endangered unionoid species in Europe (Geist 2010). *M. margaritifera* was formerly widespread and abundant, with a distributions range from the Arctic and temperate regions of Western Russia through Europe to the north-eastern seaboard of Northern America (Jungbluth et al. 1985). Several studies have revealed dramatic declines throughout its range (e.g. Bauer 1988), and the species is at present under a serious threat of extinction in Europe with only a small number of successfully recruiting populations remaining (Ziuganov et al. 1994; Young, Cosgrove and Hastie 2001; Geist and Auerswald 2007; Geist 2010). Critical factors resulting in the decline of freshwater pearl mussels are excessive pearl fishing, habitat destruction by water pollution, eutrophication, acidification, river engineering and the local decline of host fish populations (Young et al. 2001; Geist 2010).

The reproductive strategy of pearl mussel involves a larval glochidial stage, which is retained in the female brood pouch or gills and released to become a parasite on the gills of a salmonid host fish (Bauer 1994). Brown trout was found to be the most important host fish in European populations (Geist et al. 2006).

Recent studies have demonstrated that knowledge of the genetic structure of freshwater pearl mussel populations can be extremely useful for their conservation (Geist et al. 2003, 2010; Marchordom et al. 2003; Geist and Kuehn 2005, 2008; Bouza et al. 2007). Additionally, the simultaneous molecular analysis of host fish populations at the same sites can also complement an effective conservation strategy for *M. margaritifera*, because genetically unique or diverse populations of both species can be considered in conservation management (Geist and Kuehn 2005; Geist and Kuehn 2008). At the same time, the compatibility of mussel larvae with their fish hosts is important and knowledge on the genetic diversity and differentiation of the primary host, the brown trout (*Salmo trutta*) in relation to the pearl mussel therefore also needs to be considered in holistic conservation approaches. Such information is crucial both for conservation of populations in the wild as well as for captive breeding.

The objective of this study was to analyze the spatial pattern of genetic diversity and differentiation of freshwater pearl mussels and its host in the regions Brittany and Normandy (France).

Material and Methods

Sampling strategy

A total of 135 pearl mussel samples and 177 brown trout samples were collected from five French drainage systems of Aulne (one population), Blavet (two populations), Orne (one population), Loire (one population) and Sienne (one population). Pearl mussels were exclusively sampled in a way that neither harms the specimen nor has any impact on the remaining populations, namely through haemolymph sampling as described in Geist and Kuehn (2005). The sampling was carried out by J. Geist and fin clip samples from brown trout stored in EtOH (96%) were provided by M. Capoulade. For comparison and for statistical analysis (e.g. neighbour-joining (NJ) phenogram) molecular data of three Central and one North European brown trout and pearl mussel populations from the Donau (D), Elbe (D), Weser (D) and Kemijoki (FIN) catchment were also included.

DNA isolation and microsatellite analyses

Total DNA from fin clips was extracted using NucleoSpin Tissue-Kit (Macherey-Nagel), following the manufacturer's instructions for preparation of tissue material. Haemolymph samples were transferred to 1.7 mL Eppendorf vials, cooled at 5°C and processed immediately in the laboratory. After centrifugation at 14 000 g for 5 min, the supernatant was discarded and DNA was isolated from the remaining cellular pellet with the NucleoSpin Tissue Kit (Machery-Nagel), as described for the tissue samples.
Nine microsatellite loci (MarMa2671, MarMa3050, MarMa3621, MarMa4143, MarMa4322, MarMa4726, MarMa5167, MarMa5280 and MarMa5023) as described in Geist et al. (2003) and Geist and Kuehn (2008) were used for genetic analyses of *Margaritifera margaritifera*. For brown trout, 10 microsatellite loci were selected based on the recommendations of the European TroutConcert Project (www.qub.ac.uk/bb/prodohl/TroutConcert/TroutConcert. htm): loci Str15INRA,

Str60INRA, Str73INRA (Estoup et al. 1993), OmyFgt1TUF (Sakamoto et al. 1994), Str85INRA, Str543INRA (Presa and Guyomard 1996), BS131, Ssa171, Ssa85 (O'Reilly et al. 1996) and Str43INRA (Estoup et al. 1998) following the procedure described in Geist & Kuehn (2008).

Polymerase Chain Reactions (PCRs) were performed in a total volume of 12.5 µL with the following components: 25 ng of genomic DNA, 200 nM of each primer (Biomers), 0.2 mM of each dNTP, 3 mM MgCl2 (2mM MgCl2 for locus MarMa5280 and for all brown trout loci except for Str85 and Omyfgt1), 1 × PCR buffer (10 mM Tris-HCl, 50 mM KCl, 0.08% Nonidet P40), and 0.25 U *Taq* DNA Polymerase (Qbiogene). The forward primers were labelled with the fluorescent dye Cy5. PCR was carried out on a Mastercycler gradient thermal cycler (Eppendorf) under conditions as described in Geist et al. (2003) and Geist and Kuehn (2008).

PCR products were separated on 5% denaturing 19:1 acrylamide:bisacrylamide gels on an ALFexpressII DNA analyser and scored with ALLELELINKS 1.02 software (Amersham Parmacia Biotech). Electrophoresis was carried out with two internal standards in each lane. Additionally, an external standard and a previously sequenced reference sample were included on each gel in order to ensure exact scoring and to facilitate cross-referencing among gels.

Statistical and population genetic analyses

GENEPOP version 4.0 (Rousset 2008) was used to calculate allele frequencies, average allele numbers per locus (*A*), expected and observed heterozygosities (H_E, H_O), to test the genotypic distribution for conformance with Hardy–Weinberg (*HW*) expectations, to test the loci for genotypic disequilibrium and to estimate the genetic differentiation (F_{ST} according to Weir and Cockerham 1984) between pairs of populations. Tests for significant population differentiation among all pairs of populations were performed with the same software using 100,000 iterations and 1,000 de-memorisation steps (Raymond and Rousset 1995). Allelic richness (A_R) as a standardized measure of the number of alleles per locus corrected by the sample size was calculated with the software FSTAT version 2.9.3 (Goudet 2001). Alleles were considered private if they showed a frequency higher than 5% in one population and did not occur in any other population. Genetic distances between populations were

estimated using Nei D_A genetic distance (Nei et al. 1983) as implemented in the DISPAN program (Ota 1993). The resulting distance matrix was used to construct a neighbour-joining (NJ) phenogram (Saitou and Nei 1987) in MEGA version 6 (Tamura et al. 2013). Bootstrap values were calculated by generating 1000 distance matrices with DISPAN (Ota 1993). A Mantel test with 1000 iterations implemented in genalex 6 (Peakall and Smouse 2006) was used to test for correlation between pairwise values of differentiation of pearl mussel and brown trout. In order to visualize the genetic structure of both species a Discriminant Analysis of Principal Components (DAPC) implemented in the R-package adegenet (Jombart 2008; Jombart et al. 2010) for R v. 2.12. (R Development Core Team 2011) was done. The three-dimensional vectors of the DAPC can be visualized by using RedGreenBlue colour coding (Jombart 2008; Jombart et al. 2010). Equal colours characterize the same genetic constitution of an individual.

Results

Genetic differentiation of brown trout and pearl mussel

Based on F_{ST}- and D_A-values pearl mussel populations revealed a stronger degree of differentiation than brown trout populations from the same sampling sites (Fig. 1, Table 1 and Table 2). The NJ phenogram in Figure 1 illustrates the genetic structuring of both species based on Nei D_A (Nei et al. 1983). All brown trout populations with the exception of the North European population PI are closely clustered, mirroring a weak to moderate population genetic differentiation. In contrast, pearl mussel populations are separated with long-branch lengths indicating a stronger genetic differentiation. This result is supported by the visualisation of the individual genetic constitution in Figure 2 and Figure 3. As previously found in other European drainages, the regional genetic pattern of populations in the regions Normandy and Brittany does not match with drainages (e.g. BC and LO).

Mean F_{ST} for pearl mussel was 0.514, which is more than three times the value of mean F_{ST} in brown trout (0.155). F_{ST}-values among pearl mussel populations ranged from 0.055 between the two populations AI and LO from the Sienne and Blavet drainage to 0.849 between the EL and the SO population (Aulne and Loire drainage).

In brown trout, F_{ST} -values ranged from 0.041 between the KO and RV population from the Elbe and Orne drainage to 0.412 between the populations PI and LO (Kemijoki and Blavet drainage). The Mantel test found no significant correlation between the overall F_{ST} distance matrices of both species.

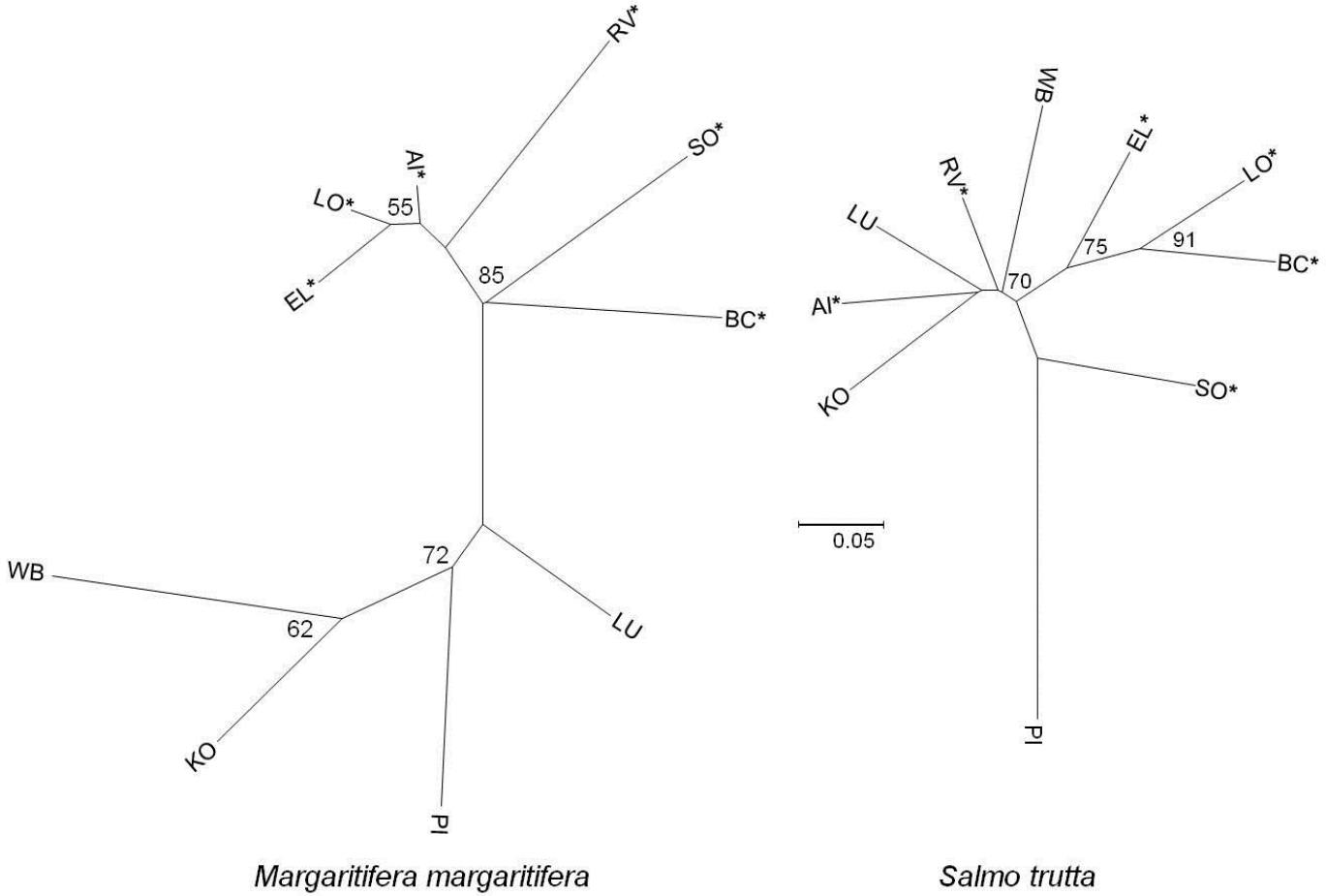

Fig. 1 Neighbour-joining (NJ) phenograms based on Nei D_A (Nei et al. 1983) genetic distance of parasitic freshwater pearl mussels (left) and their host fish, brown trout (right). The size bar (0.05) refers to both graphs. Numbers indicate nodes with bootstrap support of more than 50% for 1000 replications. French populations (AI, BC, EL, LO, SO and RV) of both species are labbeled with a black star

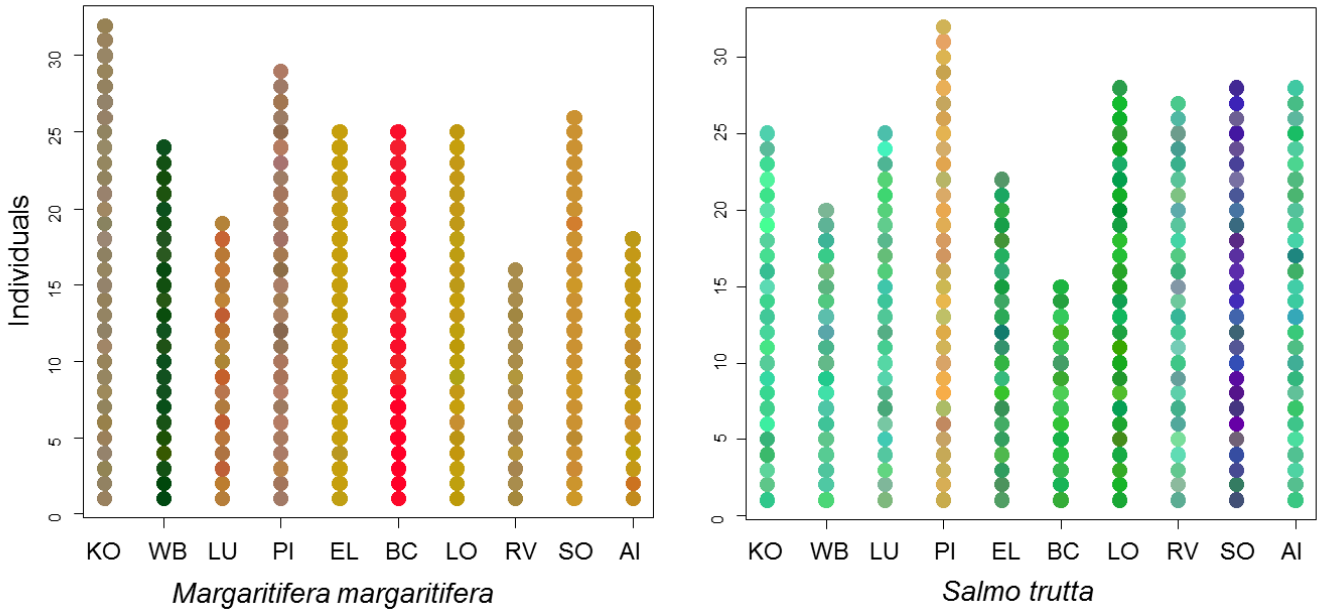

Fig. 2 Visualisation of the individual genetic constitution per population (all populations) based on the three-dimensional vectors of the DAPC and RedGreenBlue colour transformation (Jombart 2008; Jombart et al. 2010). Equal colours characterize the same genetic constitution of an individual.

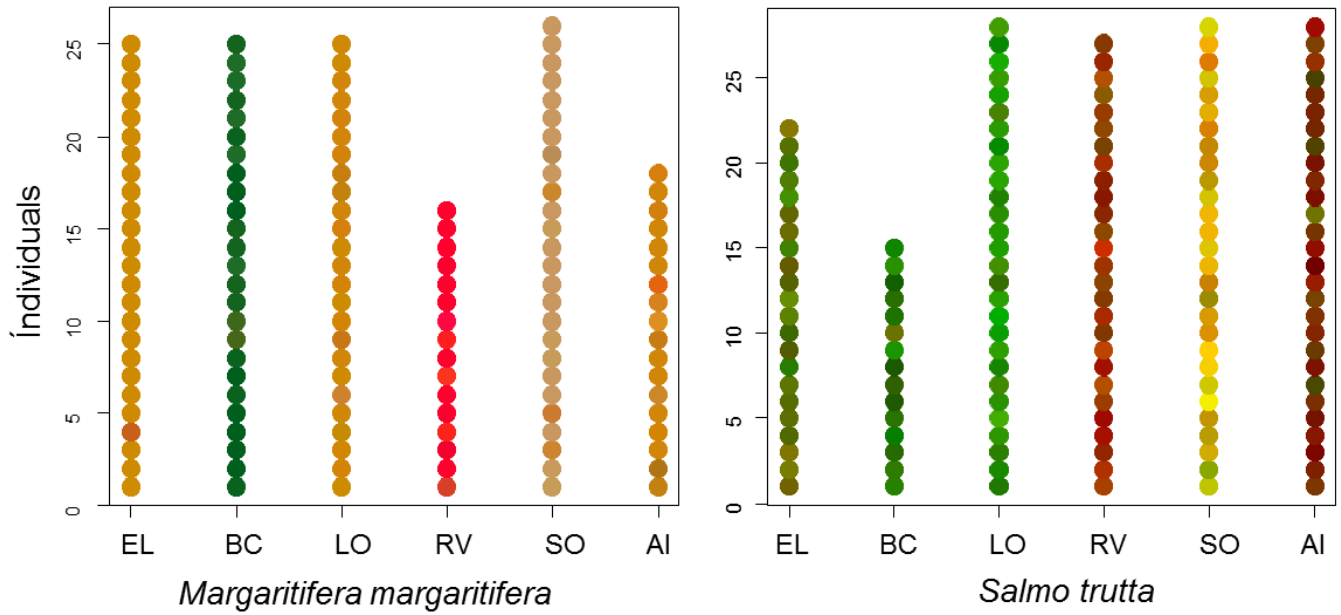

Fig. 3 Visualisation of the individual genetic constitution of Brittany and Normandy populations based on the three-dimensional vectors of the DAPC and RedGreenBlue colour transformation (Jombart 2008; Jombart et al. 2010). Equal colours characterize the same genetic constitution of an individual.

Genetic diversity in brown trout and pearl mussel

In total, higher degree of genetic diversity, as measured by average number of alleles per locus, allelic richness and heterozygosity values, was observed in brown trout populations compared to pearl mussel populations (Table 3). Minimum values for genetic diversity were particularly different between the two species. The observed heterozygosity ranged between 0.379 and 0.636 in brown trout, but only between 0.009 and 0.484 in pearl mussel. Allelic richness (A_R) varied between 1.2 (pearl mussel population EL) and 4.0 (pearl mussel population PI) and between 2.9 (brown trout population PI) and 6.3 (brown trout population KO). Among pearl mussel populations, allelic richness, mean number of alleles, as well as expected and observed heterozygosities were remarkably low in populations from Normandy and Brittany compared to all other European populations. Additionally, the highest F_{IS} values were detected in these populations, indicating a stronger degree of heterozygote deficiency. On the contrary, H_O H_E and F_{IS}-values of all brown trout populations showed little differences. A summary of the microsatellite diversity indices of both species is provided in Table 3.

Significant deviations from expected Hardy-Weinberg proportions were detected in four populations of the regions Brittany and Normandy at one (BC, LO) and two (AI, SO) loci after Bonferroni correction.

The test for genotypic disequilibrium for each pair of the nine pearl mussel and ten brown trout microsatellite loci over all populations showed no significant value after Bonferroni correction for multiple tests (P <0.00138).

Table 1 Pairwise estimates of F_{ST} values according to Weir and Cockerham (1984) between pearl mussel (*Margaritifera margaritifera*) populations (below diagonal; values $P < 0.001$ in bold) and Nei D_A (Nei et al. 1983) distances (above diagonal); $P < 0.001$

	KO	WB	LU	PI	EL	BC	LO	RV	SO	AI
KO		0.286	0.234	0.226	0.438	0.465	0.429	0.395	0.424	0.379
WB	**0.296**		0.367	0.327	0.489	0.500	0.445	0.444	0.507	0.446
LU	**0.246**	**0.421**		0.220	0.331	0.287	0.318	0.381	0.332	0.243
PI	**0.208**	**0.327**	**0.192**		0.409	0.411	0.378	0.370	0.349	0.357
EL	**0.604**	**0.759**	**0.571**	**0.527**		0.208	0.069	0.194	0.255	0.101
BC	**0.550**	**0.691**	**0.453**	**0.464**	**0.755**		0.226	0.297	0.256	0.209
LO	**0.505**	**0.634**	**0.426**	**0.426**	**0.271**	**0.534**		0.189	0.197	0.038
RV	**0.477**	**0.631**	**0.516**	**0.422**	**0.759**	**0.701**	**0.542**		0.316	0.182
SO	**0.561**	**0.738**	**0.541**	**0.440**	**0.849**	**0.736**	**0.628**	**0.777**		0.174
AI	**0.451**	**0.608**	**0.347**	**0.382**	**0.461**	**0.509**	**0.055**	**0.567**	**0.580**	

Table 2 Pairwise estimates of F_{ST} values according to Weir and Cockerham (1984) between brwon trout (*Salmo trutta*) populations (below diagonal; values $P < 0.001$ in bold) and Nei D_A (Nei et al. 1983) distances (above diagonal)

	KO	WB	LU	PI	EL	BC	LO	RV	SO	AI
KO		0.236	0.177	0.403	0.224	0.253	0.255	0.162	0.264	0.180
WB	**0.075**		0.204	0.365	0.280	0.290	0.257	0.176	0.255	0.209
LU	**0.085**	**0.101**		0.312	0.218	0.276	0.246	0.144	0.261	0.157
PI	**0.270**	**0.283**	**0.273**		0.378	0.409	0.490	0.311	0.317	0.401
EL	**0.100**	**0.139**	**0.114**	**0.305**		0.207	0.201	0.198	0.210	0.223
BC	**0.123**	**0.137**	**0.147**	**0.349**	**0.083**		0.158	0.278	0.302	0.259
LO	**0.168**	**0.138**	**0.178**	**0.412**	**0.138**	**0.053**		0.260	0.287	0.223
RV	**0.041**	**0.054**	**0.064**	**0.236**	**0.099**	**0.130**	**0.168**		0.196	0.161
SO	**0.116**	**0.143**	**0.169**	**0.285**	**0.142**	**0.173**	**0.230**	**0.090**		0.236
AI	**0.077**	**0.088**	**0.065**	**0.320**	**0.117**	**0.144**	**0.157**	**0.066**	**0.143**	

Table 3 Microsatellite diversity indices of pearl mussel (*Margaritifera margaritifera*) and brown trout (*Salmo trutta* m. *fario*) populations; ***N***, sample size; ***A***, average number of alleles per locus; A_p, number of private alleles, divided into all observed private alleles ($A_{P(TOT)}$) and those for the six populations from Brittany and Normandy ($A_{P(FR)}$); A_R, mean allelic richness; H_E, expected and H_O, observed heterozygosity; F_{IS}, value per population and result of Hardy-Weinberg probability test (***HW***)

					Salmo trutta								***Margaritifera margaritifera***							
Drainage	***Pop.***	***Code***	***Lat.***	***Long.***	***N***	***A***	$A_{P(TOT)}$	$A_{P(FR)}$	A_R	H_E	H_O	F_{IS}	***N***	***A***	$A_{P(TOT)}$	$A_{P(FR)}$	A_R	H_E	H_O	F_{IS}
Danube	Kleine Ohe	KO	48.718370	13.293300	25	7.2	1		6.3	0.694	0.616	0.114	32	2.9	2		2.7	0.424	0.369	0.131
Elbe	Wolfsbach	WB	50.316770	12.127970	20	5	2		4.7	0.628	0.633	-0.008	24	1.9	1		1.8	0.254	0.245	0.034
Weser	Lutter	LU	52.659480	10.297330	25	5.9	1		5.2	0.630	0.636	-0.009	19	2.6	0		2.5	0.393	0.412	-0.05
Kemijoki	Pikku-Luiro	PI	68.252990	28.039640	32	3.2	0		2.9	0.402	0.406	-0.011	29	4.6	6		4.0	0.502	0.484	0.036
Aulne	l´Elez	EL	48.338410	-3.818260	22	5.1	2	2	4.8	0.602	0.558	0.076	25	1.3	0	0	1.2	0.018	0.009	0.5
Blavet	le Bonne Chère	BC	48.063167	-3.125667	15	4.7	1	4	4.7	0.547	0.56	-0.024	25	1.6	0	2	1.5	0.095	0.062	0.349
	le Loc'h	LO	48.366367	-3.266333	28	5.3	0	0	4.5	0.481	0.379	0.214	25	1.8	0	0	1.7	0.164	0.049	0.707
Orne	la Rouvre	RV	48.817067	-0.391817	27	6.5	0	2	5.6	0.658	0.595	0.098	16	1.9	1	1	1.9	0.123	0.035	0.725
Loire	le Sarthon	SO	48.483183	-0.046383	28	5.1	0	2	4.4	0.542	0.514	0.052	26	1.6	1	1	1.5	0.073	0.017	0.766
Sienne	l´Airou	AI	48.897850	-1.387267	28	7.1	1	2	5.8	0.640	0.596	0.069	18	2.0	0	0	2.0	0.174	0.049	0.723
Total overall populations (mean/standard deviation)					**25**	**5.5/ 1.2**			**4.9/ 0.9**	**0.582/ 0.089**	**0.5493/ 0.091**	**0.057/ 0.074**	**23.9**	**2.2/ 1.0**			**2.1/ 0.8**	**0.222/ 0.165**	**0.173/ 0.186**	**0.392/ 0.332**

Discussion

Genetic differentiation and diversity in brown trout and pearl mussel

The degree of genetic differentiation as well as the genetic diversity of brown trout and pearl mussel was considerable different, with pearl mussel revealing a more pronounced population structure and a lower genetic variability compared to its host, the brown trout. The absence of a correlation between F_{ST} matrices of both species and the overall stronger degree of population differentiation in pearl mussels can be explained by differences in the life-history strategies. The high degree of specialization of pearl mussels to extremely oligotrophic conditions and the passive migration via host fish, probably leads to stronger isolation and fragmentation of populations compared to brown trout. Since female mussels are able to switch to simultaneous hermaphrodism (Bauer 1987) and the high number of progeny of individuals (several million glochidia larvae per year) (Young and Williams 1984b; Hastie and Young 2003) founder effects and inbreeding, resulting in loss of genetic variability, are more likely to happen in pearl mussel. In addition, the more specific habitat requirements in pearl mussel, particularly in the juvenile stages, as well as the ongoing population bottlenecks due to deficient reproduction may contribute to more pronounced genetic drift. In comparison, brown trouts have separate sexes and trout females can only produce about 1500 eggs per kg body mass (Muus and Dahlström 1981). These differences in the genetic pattern structure of European pearl mussel and brown trout populations have been previously described (Geist and Kuehn 2008).

The genetic differentiation among populations of pearl mussel in the regions Brittany and Normandy were largely independent from present-day drainage systems as previously also observed in other regions (Geist & Kuehn 2005; Geist et al. 2010). Reasons for this observation can be seen in the colonization history which does not always reflect the present day drainage systems, but also other factors such as population demographic effects (founder and drift effects) and anthropogenic factors. In the case of the generally weaker genetic differentiation of brown trout, the greater mobility of trout along with possible fish movemenets and/or stocking effects may play a role.

Management implications

A sound conservation management of pearl mussels and their host fishes requires the consideration of genetic diversity and differentiation of both species to retain a maximum of the genetic-evolutionary potential, facilitating adaptation to changing environmental conditions. In case of the freshwater pearl mussel, the pronounced genetic differentiation within populations from Normandy and Brittany suggest that this structuring should also be considered in maintaining those separate populations. On the other hand, the very low genetic variability also suggest that genetic drift or founder effects seem to have played an important role in shaping this population structure. Consequently, avoiding a further loss of rare alleles or a further decrease of genetic variation should be a priority, e.g. by the wise selection and rotation of parent mussels for the artificial breeding programm in different years with subsequent monitoring. In case of the brown trout, the predominantly moderate genetic structuring suggests that a differentiated management of stock units in this area is not mandatory. Consequently, it may be sufficient from a genetic point of view to use the same brown trout stock for the rearing of the different mussel units.

Literature

Bauer G (1987) Reproductive strategy of the freshwater pearl mussel *Margaritifera margaritifera*. *Journal of Animal Ecology* 56:691-704

Bauer G (1988) Threats to the freshwater pearl mussel *Margaritifera margaritifera* in central Europe. *Biological Conservation* 45:239-253

Bauer G (1992) Variation in the life span and size of the freshwater pearl mussel. *Journal of Animal Ecology* 61:425-436

Bouza C, Castro J, Martínez P, Amaro R, Fernández C, Ondina P, Outeiro A, San Miguel E (2007) Threatened freshwater pearl mussel Margaritifera margaritifera L. in NW Spain: low and very structured genetic variation in southern peripheral populations assessed using microsatellite markers. *Conservation Genetics* 8; 937-948

Estoup A, Presa P, Krieg F, Vaiman D, Guyomard R (1993) (CT)n and (GT)n microsatellites: a new class of genetic markers for *Salmo trutta* L. (brown trout). *Heredity* 71; 488–496

Estoup A, Rousset F, Michalakis Y, Cornuet J-M, Adriamanga M, Guyomard R (1998) Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentiation in brown trout (*Salmo trutta*). *Molecular Ecology* 7; 339–353

Geist J, Rottmann O, Schröder W, Kühn R (2003) Development of microsatellite markers for the endangered freshwater pearl mussel *Margaritifera margaritifera* L. (Bivalvia: Unionoidea). *Molecular Ecology Notes* 3; 444-446

Geist J, Kuehn R (2005) Genetic diversity and differentiation of central European freshwater pearl mussel (*Margaritifera margaritifera* L.) Populations: implications for conservation and management. *Molecular Ecology* 14; 425-439

Geist J, Porkka M, Kuehn R (2006) The status of host fish populations and fish species richness in European freshwater pearl mussel (*Margaritifera margaritifera* L.) streams; *Aquatic Conservation: Marine and Freshwater Ecosystems* 16;251-266

Geist J, Auerswald K (2007) Physicochemical stream bed characteristics and recruitment of the freshwater pearl mussel (*Margaritifera margaritifera*). *Freshwater Biology* 55; 2299-2316

Geist J, Kuehn R (2008) Host-parasite interactions in oligotrophic stream ecosystems: the roles of life history strategy and ecological niche. *Molecular Ecology* 17; 997-1008

Geist J, Söderberg H, Karlberg A, Kuehn R (2010) Drainage-independent genetic structure and high genetic diversity of endangered freshwater pearl mussels (Margaritifera margaritifera) in northern Europe. *Conservation Genetics* 11; 1339-1350

Geist J (2010) Strategies for the conservation of endangered freshwater pearl mussels (*Margaritifera margaritifera*): A synthesis of Conservation Genetics and Ecology. *Hydrobiologia* 644; 69-88

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). URL: www.unil.ch/ izea/softwares/fstat.html

Hastie LC, Young MR (2003) Conservation of the freshwater pearl mussel 2. relationship with salmonids. Conserving Natura 2000 *Rivers Conservation Techniques Series* No. 3. English Nature, Peterborough.

Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24; 1403–1405

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* 11; 94

Jungbluth JR, Coomans HE, Groh H (1985) Bibliographie der *Flussperlmuschel Margaritifera margaritifera* (Linn. 1785). Verslagenen Technische Gegevens No 41 Institut voor Taxonomische Zoologie, Universiteit Amsterdam

Marchordom A, Araujo R, Erpenbeck D, Ramos MA (2003) Phylogeography and conservation genetics of the endangered European Margaritiferidae (Bivalvia: Unionoidea). *Biological Journal Linnean Society* 78;235-252

Muus BJ, Dahlström P (1981) Süßwasserfische Europas — Biologie, Fang, wirtschaftliche Bedeutung; BLV Bestimmungsbuch; 5. Aufl.; BLV Verlagsgesellschaft, München.

Nei M, Tajima F, Tateno Y (1983) Accuracy of genetic distances and phylogenetic trees from molecular data. *Journal of Molecular Evolution* 19; 153-170

Ota T (1993) DISPAN: Genetic Distance and Phylogenetic Analyses Software. Pennsylvania State University, Pennsylvania.

O'Reilly PT, Hamilton LC, McConnell SK, Wright JM (1996) Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. *Canadian Journal of Fisheries and Aquatic Sciences* 53; 2292–2298

Peakall R, Smouse PE (2006) genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6; 288–295

Presa P, Guyomard R (1996) Conservation of microsatellites in three species of salmonids. *Journal of Fish Biology* 49; 1326–1329

Raymond M, Rousset F (1995) An exact test for population differentiation. *Evolution* 49; 1280-1283

Rousset F (2008) GenePop'007: a complete re-implementation of the GenePop software for Windows and Linux. *Molecular Ecology Resources* 8; 103-106

Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4; 406-425

Sakamoto T, Okamoto N, Ikeda Y, Nakamura Y, Sato T (1994) Dinucleotide-repeat polymorphism in DNA of rainbow trout and its application in fisheries science. *Journal of Fish Biology* 44; 1093–1096

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30; 2725-2729

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* 38; 1358-1370

Young M, Williams J (1984a) The reproductive biology of the freshwater pearl mussel in Scotland. II. Laboratory Studies. *Archiv für Hydrobiologie* 100; 29–43

Young MR, Cosgrove PJ, Hastie LC (2001) The extent of, and causes for, the decline of a highly threatened naiad: *Margaritifera margaritifera*; In: Ecology and Evolution of the freshwater mussels Unionoidea (Eds Bauer G and Wächtler K). *Ecological Studies* 145:337-357 Springer Verlag, Heidelberg

Ziuganov V, Zotin A, Nezlin L, Tretiakov V (1994) The freshwater pearl mussels and their relationships with salmonid fish. *VNIRO, Russian Federal Institute of Fisheries and Oceanography*, Moscow 104 pp.