Molecular Ecology (2004)

doi: 10.1111/j.1365-294X.2004.02420.x

Geist Kuehn 2004

Genetic diversity and differentiation of central European freshwater pearl mussel (*Margaritifera margaritifera* L.) populations: implications for conservation and management

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Abstract

Despite the fact that mollusc species play an important role in many aquatic ecosystems, little is known about their biodiversity and conservation genetics. Freshwater pearl mussel (*Margaritifera margaritifera* L.) populations are seriously declining all over Europe and a variety of conservation programs are being established to support the remaining endangered central European populations. In order to provide guidelines for conservation strategies and management programs, we investigated the genetic structure of 24 freshwater pearl mussel populations originating from five major central European drainages including Elbe, Danube, Rhine, Maas and Weser, representing the last and most important populations in this area. We present a nondestructive sampling method of haemolymph for DNA analyses, which is applicable for endangered bivalves. The analyses of nine microsatellite loci with different levels of polymorphism revealed a high degree of fragmented population structure and very different levels of genetic diversity within populations. These patterns can be explained by historical and demographic effects and have been enforced by anthropogenic activities. Even within drainages, distinct conservation units were detected, as revealed from high F_{ST} values, private alleles and genetic distance measures. Populations sampled close to contact zones between main drainage systems showed lowest levels of correct assignment to present-day drainage systems. Populations with high priority for conservation should not only be selected by means of census population size and geographical distance to other populations. Instead, detailed genetic analyses are mandatory for revealing differentiation and diversity parameters, which should be combined with ecological criteria for sustainable conservation and recovery programs.

Keywords: Bivalvia, conservation unit, fragmented population structure, microsatellites, Mollusca, noninvasive sampling

Received 12 August 2004; revision received 27 October 2004; accepted 27 October 2004

Introduction

Unionid bivalves are a diverse group of molluscs with a worldwide distribution (Roe & Hoeh 2003). They play an important role in lotic and lenitic ecosystems and their presence or absence in a lake or stream has manifold implications for aquatic ecosystems (Bauer & Wächtler 2001). Nowadays, many species suffer from severe population declines, and bivalve biodiversity is diminishing at a nearly unprecedented pace (e.g. Ricciardi & Rasmussen 1999).

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One example is the freshwater pearl mussel (*Margaritifera margaritifera* L.), an indicator species for undisturbed headwater regions and small streams, which occurred in extreme densities until the middle of the 19th century, often covering the river bottoms in one or more layers. *M. margaritifera* has declined substantially throughout its holarctic range and is now highly vulnerable or threatened with extinction almost everywhere, with few populations still having a significant number of juveniles present (Cosgrove *et al.* 2000; Young *et al.* 2001). Some authors even consider it to be among the most critically endangered freshwater mussels in the world (Marchordom *et al.* 2003). Deterministic factors like pearl fishing, water pollution

and eutrophication, acidification, habitat destruction, river engineering, and the decline of host fish populations, have all more or less contributed to the decline of freshwater mussels. Small isolated populations, in turn, are more susceptible to the effects of inbreeding and genetic drift, which can result in reduced adaptability, survival and reproduction. Nowadays, only few populations still exist in central Europe, mainly in the Elbe and Danube drainages, and some smaller relict populations in the Rhine/Main, Maas and Weser drainages. Pearl mussels can reach an age of more than 100 years (Bauer 1992) and most of these populations have not been reproducing for the past 30–50 years.

The species is restricted to habitats with flowing waters which are low in lime and nutrients, and requires special conditions to complete its complex life cycle. Freshwater pearl mussels have separate sexes, with females being able to switch to hermaphrodites at low population densities (Bauer 1987). Like all freshwater mussels (Unionoidea), pearl mussels have a reproductive strategy that involves a larval 'glochidia' stage, which is retained in the female brood pouch or gills and released for their intermediate stage as a parasite on a host fish before transforming into bottom-dwelling juveniles. Suitable host fishes for freshwater pearl mussels are only salmonids, with a preference for brown trout (*Salmo trutta* f. *fario*) in central European populations (Wächtler *et al.* 2001).

The vulnerability of the species requires conservation, recovery and management strategies, which include investigation of current levels of genetic diversity and differentiation within and between populations as a basis for sustainable management recommendations. Genetic studies on bivalves based on conchological convergences and parallelisms in shell shape and external morphology can be highly influenced by environmental variables such as substrate composition or water velocity (e.g. Johnson 1970; Watters 1994). Available allozymes and mitochondrial genes were found not to resolve genetic structures beyond species level for freshwater pearl mussels (Nagel & Badino 2001; Marchordom *et al.* 2003). Therefore, we developed species-specific microsatellite markers for freshwater pearl mussels (Geist *et al.* 2003). Nine microsatellite markers were used in this study to reveal population diversity and differentiation among 24 central European freshwater pearl mussel populations of the five major drainages of Elbe, Danube, Rhine, Maas and Weser as a basis for ongoing species conservation efforts in these areas. The intended recovery strategies, based on semiartificial infections of host fish, supportive breeding and the use of cultured unionids as a conservation tool underscores the need to recognize the genetic composition of natural and managed populations. To our knowledge, this is the first study on population and conservation genetics of a European freshwater bivalve, applying microsatellite markers.

Materials and methods

Sampling strategy

A total of 558 individuals from 24 pearl mussel populations originating from five central European main drainage systems of Elbe (8 populations), Danube (8 populations), Rhine (4 populations), Maas (2 populations) and Weser (2 populations) were included in this study, representing the most important remaining pearl mussel populations of Austria, Belgium, the Czech Republic, Germany and Luxembourg (Fig. 1). A geographically isolated relict

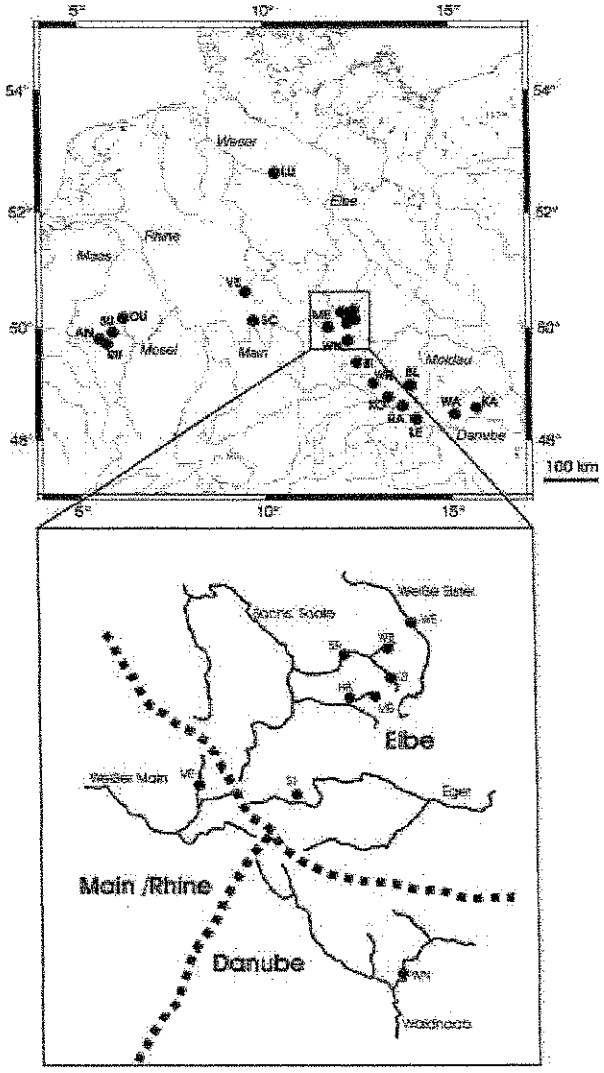

Fig. 1 Sampling locations (black circles) of freshwater pearl mussel (*Margaritifera margaritifera* L.) populations in central Europe and magnification of the sampling sites at the contact zone between the three main drainage systems of Elbe, Main/Rhine and Danube; sample codes according to Table 1.

Table 1 Samples used for genetic analyses; N_c = estimates for census population sizes counted 1998–2003; *indicates small sample size as a result of small census population or sampling restrictions but expected to be representative for remaining population; A = Austria, B = Belgium, CZ = Czech Republic, D = Germany, L = Luxembourg

Drainage	Subdrainage	Population	Code	Country	N_c	Sample size
Elbe	Sächsische Saale	Zinnbach	ZI	D	7000	26
	Sächsische Saale	Südliche Regnitz	SR	D	13000	25
	Sächsische Saale	Wolfsbach	WB	D	2100	24
	Sächsische Saale	Höllbach	HB	D	34000	25
	Sächsische Saale	Mähringsbach	MB	D	11000	25
	Sächsische Saale	Weiße Elster (Triebelbach and Rauner Bach)	WE	D	< 50	6*
	Eger→Sächsische Saale	Steinselb	ST	D	16	16
	Moldau	Blanice	BL	CZ	50000	33
Danube	Naab	Waldnaab	WN	D	3000	26
	Naab	Biberbach	BI	D	500	25
	Regen	Wolfertsrieder Bach	WR	D	2000	21
	Gaißa	Kleine Ohe	KO	D	7000	32
		Ranna	RA	D	600	29
	Aschach	Leitenbach	LE	A	500	24
	Aist	Waldaist	WA	A	18000	24
		Kamp	KA	A	23000	24
Rhine	Weißer Main→Main	Metzlersreuther Bach	ME	D	50	26
	Fränkische Saale→Main	Schondra	SC	D	100	20
	Sauer→Mosel	Our	OU	L, D, B	1350	27
	Mosel	Sauer	SU	B	250	26
Maas	Semois	Anlier	AN	B	1400	26
	Semois	Rulles	RU	B	300	25
Weser	Aller	Lutter	LU	D	4200	19
	Fulda	Vogelsberg (Ellersbach, Altefeld)	VB	D	4	4*

population (Vogelsberg, VB) and a population for which an artificial culturing technique is currently being established (Weiße Elster, WE), were also included in this study despite the fact that they consist of a few individuals only, rendering small sample numbers (4 and 6, respectively) for analyses. A description of the sampled populations, including estimates for their current census population sizes, is provided in Table 1. For species protection reasons it is not allowed to provide detailed GPS-coordinates, but they can be made available on demand to the corresponding author. Most pearl mussel populations are in danger of extinction, which implies the use of a sampling method that has no negative impacts on the extant populations. Two principal sources were used for DNA extraction in this study: sampling of dead individuals found during river surveys (10% of samples) and sampling of haemolymph from living specimens (90% of samples). For the latter method, mussels were removed from the river bottom and approximately 0.1–0.3 mL of haemolymph was collected with 1 mL syringes attached to 0.80 × 50 mm 21G × 2″ sterican needles by gently inserting the needle into the foot of the mussels. Shells of sampled specimens were cleaned with paper towels and marked with white waterproof paint for later inspection. All mussels were then returned to their original locations within the river bed substrate. Inspection of 250 sampled mussels from 10 populations after 4 weeks, 6 months and 1 year revealed no mortality caused by the sampling method. Special attention was attributed to representative sample collection, including samples from a long river stretch in the range of mussel distribution within each river and including samples of mussels from all age classes except those with a size smaller than 4.5 cm (approximately corresponding to an age of max. 20 years). However, such young mussels only occurred in two of the investigated rivers in central Europe, from which dead individuals from younger age classes were available and included into the analyses. The sample collection was carried out from 2001 to 2003.

DNA isolation and microsatellite analyses

From dead specimens, total DNA was extracted from foot and adductor mussel tissue 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 14000 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.

A total of nine microsatellite loci with different levels of polymorphism were selected for this study: eight loci (MarMa2671, MarMa3050, MarMa3621, MarMa4143, MarMa4322, MarMa4726, MarMa5167 and MarMa5280) previously described in Geist *et al.* (2003), and one additionally developed locus MarMa5023 (GenBank accession no. AY633928). 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, 0.2 mM of each dNTP, 3 mM $MgCl_2$ (2 mM $MgCl_2$ for locus MarMa5280), 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 end-labelled with the fluorescent dye Cy5. PCR was carried out on a Mastercycler Gradient thermal cycler (Eppendorf) under the conditions described by Geist *et al.* (2003). Annealing temperature was 55 °C for locus MarMa5023. PCR products were separated on 5% denaturing 19 : 1 acrylamide:bisacrylamide gels on 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 3.3 (Raymond & Rousset 1995a) 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, to calculate pairwise F_{ST} values and to test the significance of allelic differentiation. Allelic richness (A_R) as a standardized measure of the number of alleles per locus corrected by the sample size was calculated with the FSTAT version 2.9.3 program package (Goudet 2001). FSTAT version 2.9.3 was also used to test for differences between drainages (1000 permutations, two-sided test). Alleles were deemed as private alleles if they showed a frequency of more 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 in MEGA version 2 (Kumar *et al.* 1993). Bootstrap analysis was performed by first generating 1000 distance matrices which were then used to generate 1000 neighbour-joining trees in DISPAN (Ota 1993). ARLEQUIN 2.0 software (Schneider *et al.* 2000) was used to hierarchically quantify genetic population structure by analysis of molecular variance (AMOVA; Excoffier *et al.* 1992), and to incorporate molecular information based on allelic frequencies. All probability tests were performed applying the Markov chain algorithm (Guo & Thompson 1992; Raymond & Rousset 1995b). Sequential Bonferroni adjustments (Rice 1989) were used to correct for multiple tests. The Bayesian approach of population assignment test (Cornuet *et al.* 1999; 'as it is' option) implemented in the GENECLASS 1.0.02 program (Piry & Cornuet 1999) was used to estimate the likelihood of an individual's multilocus genotype to be assigned to the population from which it was sampled.

Relatedness between individuals was estimated based on the F value from the 2MOD program (Ciofi & Bruford 1999) which refers to the probability that two genes share a common ancestor within a population and correlates with effective population sizes. The 2MOD program was also used to investigate the population history of the central European freshwater pearl mussel populations based on the coalescent theory. The method uses the comparison of the relative likelihoods of a model of immigration-drift equilibrium (gene flow model) vs. drift since a certain time. A Markov chain Monte Carlo simulation (100 000 iterations) was computed, and the first 10% of the output was discarded in order to avoid bias resulting from the starting conditions.

Additionally, populations were tested for recent reduction of their effective population size based on the approach of Cornuet & Luikart (1996) with the program BOTTLENECK (Piry *et al.* 1999). The Wilcoxon sign-rank test was used to test the significance of heterozygote excess under three different models, the infinite allele model (IAM), the stepwise mutation model (SMM) and the two-phase model (TPM) with 5% multistep changes and variance of 12, following the recommendations of Piry *et al.* (1999).

The heterozygosity contribution (CT) of each population to total diversity was calculated with the CONTRIB program (Petit *et al.* 1998) by separately calculating diversity and differentiation indices measured by the expected heterozygosity. This approach allows a simultaneous comparison of populations with the average values over all populations by visualizing positive or negative CT percentage — values and supplements the genetic characterization of populations and the selection of priority populations for conservation.

Results

Linkage and Hardy–Weinberg equilibrium

The test for genotypic disequilibrium for each pair of the nine microsatellite loci over all populations gave two significant values ($P < 0.05$) for 36 comparisons (two significant

Table 2 Microsatellite diversity indices for central European freshwater pearl mussel (*Margaritifera margaritifera* L.) populations. Sample size (N), average number of alles per locus (A), mean allelic richness per population (A_R), number of private alleles (A_P), frequency of private alleles (f_P), expected (H_E) and observed (H_O) heterozygosity, result of Hardy–Weinberg probability test for deviation from expected Hardy–Weinberg proportions (P_{HW}), F value based on the 2MOD programme, and test of heterozygosity excess (HE) using Wilcoxon sign-rank test based on infinite allele model (IAM), two-phased model (TPM) and stepwise mutation model (SMM)

Population	N	A	A_R	A_P	f_P	H_E	H_O	P_{HW}	F	$HE_{(IAM/TPM/SMM)}$
Elbe										
ZI	26	2.9	1.8	—		0.381	0.372	n.s.	0.299	+/+/−
SR	25	3.0	1.8	—		0.393	0.400	n.s.	0.295	−/−/−
WB	24	1.9	1.5	—		0.254	0.245	n.s.	0.448	−/−/−
HB	25	3.6	2.0	—		0.448	0.418	n.s.	0.156	+/+/−
MB	25	3.7	1.9	—		0.441	0.413	n.s.	0.095	+/−/−
WE	6	2.6	1.9	—		0.436	0.278	n.s.	0.133	−/−/−
ST	16	3.4	2.0	—		0.447	0.361	*	0.066	−/−/−
BL	33	4.9	2.1	1	14.06	0.485	0.418	**	0.064	−/−/−
average	22.5	3.236	1.9	0.125		0.411	0.363		0.195	
Danube										
WN	26	3.1	1.9	—		0.415	0.385	n.s.	0.164	+/+/−
BI	25	3.0	2.0	—		0.461	0.489	n.s.	0.278	+/+/−
WR	21	4.0	2.1	—		0.531	0.460	***	0.060	+/−/−
KO	32	2.9	1.9	—		0.424	0.369	n.s.	0.248	+/+/−
RA	29	3.3	2.0	—		0.479	0.494	n.s.	0.216	+/+/−
LE	24	3.7	2.0	—		0.480	0.449	n.s.	0.218	−/−/−
WA	24	2.7	1.4	—		0.176	0.163	n.s.	0.389	−/−/−
KA	24	1.1	1.0	—		0.005	0.005	n.d.	0.944	n.d.
average	*25.6*	*2.972*	*1.8*	*0.000*		*0.371*	*0.352*		*0.315*	
Rhine										
ME	26	2.1	1.6	—		0.313	0.325	n.s.	0.560	+/+/−
SC	20	1.6	1.2	1	97.50	0.081	0.023	***	0.856	n.d.
OU	27	1.8	1.3	1	11.11	0.184	0.123	n.s.	0.685	−/−/−
SU	26	1.3	1.2	—		0.082	0.038	*	0.860	n.d.
average	24.8	1.695	1.3	0.500		0.165	0.127		0.740	
Maas										
AN	26	1.7	1.2	3	98.08 78.85 12.00	0.107	0.062	***	0.656	n.d.
RU	25	1.1	1.1	—		0.052	0.044	n.d.	0.942	n.d.
average	*25.5*	*1.389*	*1.2*	*1.500*		*0.080*	*0.053*		*0.664*	
Weser										
LU	19	2.6	1.8	1	41.67	0.393	0.412	n.s.	0.385	−/−/−
VB	4	1.9	1.6	1	33.33	0.288	0.185	n.s.	0.451	−/−/−
average	11.5	2.222	1.7	1		0.341	0.299		0.418	
Total average	**23.3**	**2.7**	**1.7**			***0.323***	***0.289***		***0.395***	

values are expected by chance at the 5% level). After Bonferroni correction for multiple tests, none of the combinations remained significant at the experimental level ($P < 0.00138$). When each population was tested separately, a linkage equilibrium between all pairs of loci was generally observed, with only few exceptions: four significant values were found for the Waldaist (WA) population and one for the Mährингsbach (MB) population. Different loci were involved in these cases. Generally, this test implies that the genotypes of the loci used in this study segregated independently.

After Bonferroni correction, the probability test by the Markov chain method based on the 'exact HW test' of Haldane (1954) for each locus in each population showed only five significant deviations: populations Steinselb (ST) and Schondra (SC) at locus MarMa3621, populations WR and BL at locus MarMa4726, and population AN at locus MarMa3050.

Six populations out of 24 displayed significant deviations from the expected HW proportions after applying sequential Bonferroni correction (see Table 2). These deviations are not systematic, occur at different loci

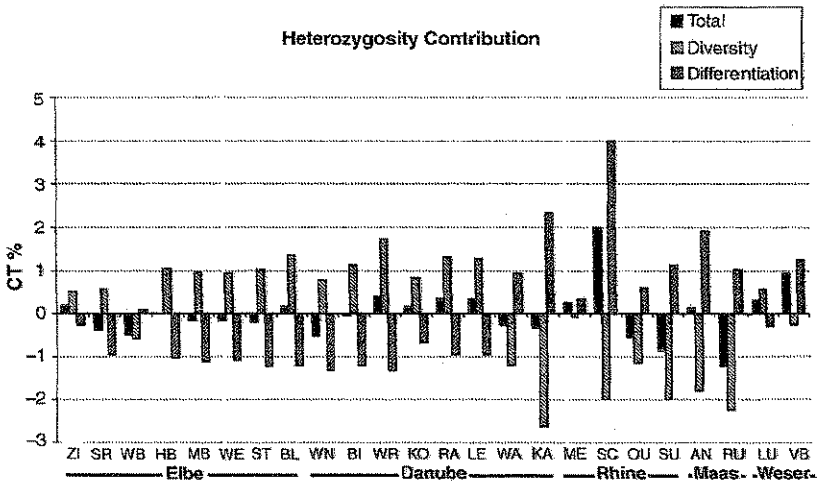

Fig. 2 Heterozygosity contribution *CT* to total diversity (subdivided into a diversity and a differentiation compound) for 24 central European freshwater pearl mussel (*Margaritifera margaritifera* L.) populations based on CONTRIB-calculations according to Petit *et al.* (1998).

(MarMa3621, MarMa4726, MarMa5167, MarMa3050 and MarMa5023) for different populations and with a maximum of two deviations in the Blanice (BL) population.

Genetic diversity and relatedness within populations

An average of 7.8 alleles (standard deviation SD = 5.3) was observed for the nine microsatellite loci applied in this study. The number of alleles per locus ranged from two at loci MarMa2671 and MarMa5280 to a maximum of 16 alleles at locus MarMa5167. Allelic variation, expressed by the average number of alleles per locus (A) and allelic richness (A_R), varied strongly between and within drainage systems and was highest in the Blanice river (BL) and Wolfertsrieder Bach (WR) from the Elbe and the Danube drainage systems, respectively. A summary of the microsatellite diversity indices is provided in Table 2. The majority of brooks and rivers from the Elbe and Danube drainage systems tend to have a higher diversity than those from the other central European pearl mussel populations, with a few exceptions. The lowest observed values for allelic diversity ($A = 1.1$; $A_R = 1.0$) were found in the Kamp (KA) from the Danube drainage system and in the generally smaller remnant populations from the Rhine and Maas drainages, where the highest values for allelic richness are 1.6 (Metzlersreuther Bach, ME) and 1.2 (Anlier, AN). Maximum values for the average number of alleles per locus and for allelic richness were found in the BL population ($A = 4.9$; $A_R = 2.1$). The expected heterozygosity (H_E) per population was between 0.005 for KA and 0.485 for BL, and the observed heterozygosity (H_O) ranged between 0.005 for KA and 0.494 for the Ranna (RA), with the average H_E being 0.323 and the average H_O being 0.289 (Table 2).

Private alleles occurred at five different loci in six different populations and usually showed high frequencies ranging from 11.11% in the Our (OU) up to 98.08% in AN. They occurred in isolated relict populations from Lutter (LU), Vogelsberg (VB) and Schondra (SC), but were also observed in drainage systems, in which other pearl mussel populations are still present. The maximum of private alleles (3) was found in AN from the Maas drainage, although it is not far from the Rulles (RU) population. One private allele was also found in the OU population, situated in the same Rhine subdrainage as the Sauer (SU) population. With exception of the highly diverse BL population, no private alleles were detected in populations which were once connected and where still a larger number of populations exist within a small geographical range (Elbe and Danube systems).

The proportion of common ancestors within each population as inferred from the F values of the 2MOD program covered an extreme range from $F = 0.060$ in WR to $F = 0.944$ and 0.942 in KA and RU, respectively. The correlation between F value and census population size is slightly negative ($r^2 = 0.05$ and $P = 0.288$). A low probability of common ancestors as revealed by the F values was not restricted to large and dense populations such as BL (census population size = 50 000, $F = 0.064$), in which lower rates of hermaphrodism and self-fertilization would be expected, but occasionally also occurred in populations like ST ($F = 0.066$), in which the total population only consisted of 16 individuals distributed over a brook section of approximately 300 m. The highest F values were found in comparatively small populations of the Rhine and Maas drainages (e.g. SC, census population size = 100, $F = 0.856$; RU, census population size = 300, $F = 0.942$) as well as in comparatively large populations (e.g. KA, census population size = 23 000, $F = 0.944$). On average, F values were lowest in populations of the Elbe drainage followed by Danube and Weser drainages.

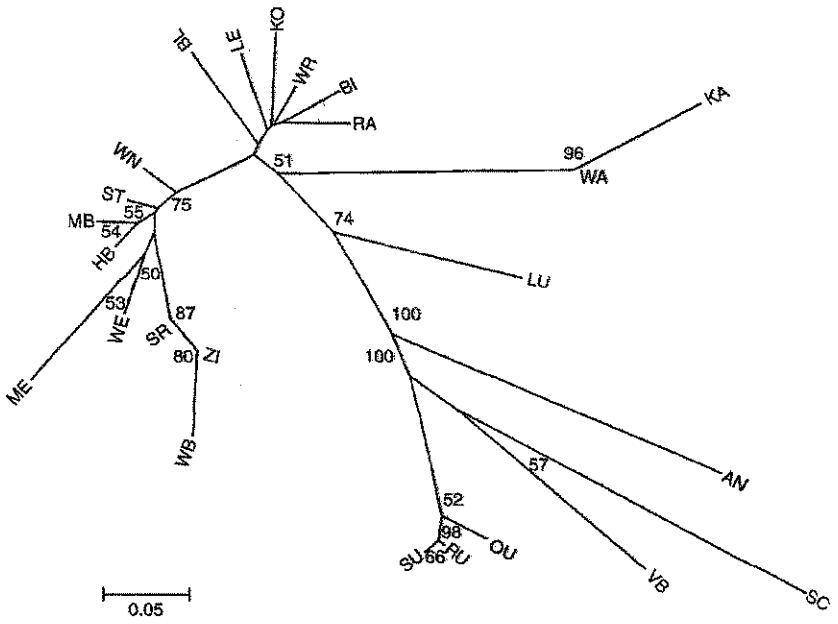

Fig. 3 Neighbour-joining (NJ) phenogram based on *Nei* D_A (Nei *et al.* 1983) genetic distance for central European freshwater pearl mussel populations. Numbers indicate nodes with bootstrap support of more than 50% for 1000 replications.

The Wilcoxon sign-rank test ($P < 0.05$) revealed evidence for recent bottlenecks in nine and seven populations, according to IAM and TPM, respectively (Table 2). Assuming an SMM, however, none of the populations revealed heterozygote excess. Five populations had less than four polymorphic microsatellite loci and could therefore not be tested.

The *CT* of each population to total diversity is visualized in Fig. 2, which demonstrates the large differences in diversity and differentiation of populations. Highest diversity contributions were observed in regions with a large number of remaining populations (Elbe and Danube systems). From the smaller populations of the Rhine, Maas and Weser catchments, only the LU population, which is still reproducing, showed a positive *CT* with respect to diversity. The two most downstream Danubian populations of WA and KA, and the small SC, SU, AN, and RU populations showed the most negative values for diversity contribution.

Genetic differentiation between populations

The microsatellite markers applied in this study reveal a high degree of genetic differentiation among most of the remaining central European freshwater pearl mussel populations with an overall average F_{ST} value of 0.374 (SD = 0.23). Pairwise F_{ST} values ranged from 0.001 between the geographically adjacent populations of Steinselb (ST) and Höllbach (HB) to values as high as 0.940 between the geographically very distant populations of RU from the Rhine drainage and KA from the most downstream Danubian pearl mussel tributary. The differences in genotype frequencies were highly significant ($P < 0.001$) for most pairwise comparisons of populations (Table 3).

F_{ST} values differ significantly ($P = 0.036$) within drainages and are on average highest for Maas ($F_{ST} = 0.773$), followed by the Rhine ($F_{ST} = 0.645$) and the Weser ($F_{ST} = 0.369$). For populations belonging to the Elbe and Danube system, F_{ST} values are comparatively low, with $F_{ST} = 0.121$ and 0.240, respectively.

AMOVA of hierarchical gene diversity revealed that 58% of the genetic variation was accounted within individuals, 5% among individuals within populations and 37% among populations. The global fixation indices were 0.079, 0.374 and 0.423 for F_{IS}, F_{ST} and F_{IT}, respectively.

The NJ phenogram depicting the underlying structure of the *Nei* D_A distance matrix illustrates the high degree of genetic differentiation between the populations, and reveals that the observed genetic structure does not necessarily match with drainages at present (Fig. 3). For instance, the AN and RU populations are quite clearly separated with long branch lengths in the NJ dendrogram, supported by high bootstrap values, despite the fact that both belong to the Maas drainage and that their geographical distance is only 20 km of river length. Danubian populations do not cluster together either, but split in a southeastern group (WA and KA), a central Danubian group (Leitenbach, LE; Kleine Ohe, KO; Wolfertsrieder Bach, WR; Biberbach, BI and Ranna, RA), and a northernmost Danubian population (Waldnaab, WN). In the contact zone of the three main drainage systems of Main/Rhine, Elbe and Danube in northern Bavaria, the separation

Table 3 Pairwise estimates of F_{ST} between central European freshwater pearl mussel (*Margaritifera margaritifera* L.) populations $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (below diagonal) and *Nei* D_A (Nei *et al.* 1983) distances (above diagonal)

	Elbe							Danube							Rhine						Maas		Weser	
Population	ZI	SR	WB	HB	MB	WE	ST	BL	WN	BI	WR	KO	RA	LE	WA	KA	ME	SC	OU	SU	AN	RU	LU	VB
ZI (Zinnbach)		0.026	0.025	0.092	0.102	0.128	0.136	0.196	0.126	0.221	0.213	0.185	0.332	0.309	0.433	0.506	0.277	0.995	0.569	0.624	0.618	0.617	0.354	0.658
SR (Südl Regnitz)	0.039 ***		0.038	0.039	0.048	0.048	0.052	0.139	0.056	0.170	0.142	0.163	0.216	0.224	0.328	0.396	0.173	0.892	0.431	0.461	0.457	0.451	0.275	0.604
WB (Wolfsbach)	0.058 ***	0.081 ***		0.146	0.135	0.144	0.173	0.226	0.167	0.205	0.254	0.246	0.394	0.339	0.330	0.386	0.377	0.870	0.552	0.609	0.532	0.593	0.418	0.674
HB (Höllbach)	0.111 ***	0.051 ***	0.210 ***		0.014	0.037	0.003	0.148	0.018	0.149	0.103	0.138	0.135	0.182	0.364	0.446	0.129	0.915	0.434	0.475	0.516	0.470	0.263	0.627
MB (Mähringsbach)	0.122 ***	0.062 ***	0.200 ***	0.016 ***		0.032	0.017	0.137	0.028	0.151	0.088	0.142	0.170	0.200	0.329	0.411	0.169	0.873	0.388	0.403	0.456	0.395	0.208	0.578
WE (Weiße Elster)	0.157 ***	0.064 **	0.252 ***	0.038 **	0.031 **		0.041	0.159	0.069	0.133	0.116	0.128	0.171	0.229	0.324	0.395	0.148	0.760	0.409	0.435	0.398	0.405	0.245	0.598
ST (Steinselb)	0.155 ***	0.067 ***	0.248 ***	0.001	0.018 **	0.032		0.099	0.006	0.148	0.084	0.151	0.117	0.136	0.375	0.469	0.100	0.783	0.333	0.371	0.461	0.369	0.203	0.542
BL (Blanice)	0.188 ***	0.142 ***	0.257 ***	0.134	0.128 ***	0.134 ***	0.092 ***		0.096	0.138	0.087	0.094	0.129	0.070	0.398	0.510	0.164	0.690	0.272	0.298	0.490	0.282	0.189	0.295
WN (Waldnaab)	0.151 ***	0.075 ***	0.238 ***	0.023 ***	0.034 ***	0.081 ***	0.006 **	0.100 ***		0.112	0.069	0.113	0.115	0.121	0.329	0.413	0.159	0.751	0.325	0.362	0.427	0.341	0.199	0.435
BI (Biberbach)	0.217 ***	0.175 ***	0.260 ***	0.143 ***	0.146 ***	0.132 ***	0.140 ***	0.124 ***	0.120 ***		0.069	0.124	0.103	0.101	0.174	0.244	0.413	0.542	0.313	0.357	0.367	0.305	0.295	0.344
WR (Wolferstr. B)	0.194 ***	0.141 ***	0.278 ***	0.094 ***	0.084 ***	0.093 ***	0.073 ***	0.074 ***	0.074 ***	0.065 ***		0.072	0.032	0.040	0.212	0.284	0.238	0.774	0.334	0.350	0.503	0.328	0.207	0.464
KO (Kleine Ohe)	0.197 ***	0.178 ***	0.296 ***	0.142 ***	0.147 ***	0.140 ***	0.151 ***	0.097 ***	0.128 ***	0.130 ***	0.075 ***		0.146	0.120	0.369	0.448	0.241	0.794	0.524	0.556	0.597	0.499	0.262	0.514
RA (Ranna)	0.273 ***	0.202 ***	0.361 ***	0.128 ***	0.155 ***	0.157 ***	0.109 ***	0.115 ***	0.119 ***	0.100 ***	0.031 ***	0.144 ***		0.045	0.288	0.371	0.210	0.835	0.310	0.329	0.531	0.304	0.249	0.467
LE (Leitenbach)	0.262 ***	0.207 ***	0.340 ***	0.161 ***	0.174 ***	0.188 ***	0.123 ***	0.066 ***	0.124 ***	0.098 ***	0.036 ***	0.122 ***	0.046 ***		0.293	0.385	0.273	0.688	0.315	0.363	0.589	0.339	0.289	0.368
WA (Waldaist)	0.477 ***	0.417 ***	0.506 ***	0.410 ***	0.395 ***	0.486 ***	0.438 ***	0.393 ***	0.405 ***	0.274 ***	0.294 ***	0.415 ***	0.345 ***	0.357 ***		0.004	0.730	0.896	0.577	0.585	0.472	0.523	0.560	0.752
KA (Kamp)	0.624 ***	0.578 ***	0.688 ***	0.564 ***	0.555 ***	0.782 ***	0.629 ***	0.534 ***	0.566 ***	0.449 ***	0.465 ***	0.557 ***	0.497 ***	0.525 ***	0.079 ***		0.856	1.026	0.703	0.710	0.533	0.638	0.666	0.920
ME (Metzlersr. B)	0.312 ***	0.227 ***	0.440 ***	0.170 ***	0.208 ***	0.218 ***	0.146 ***	0.189 ***	0.207 ***	0.353 ***	0.241 ***	0.265 ***	0.230 ***	0.274 ***	0.613 ***	0.747 ***		1.394	0.502	0.494	0.717	0.501	0.280	0.681
SC (Schondra)	0.651 ***	0.634 ***	0.725 ***	0.603 ***	0.601 ***	0.713 ***	0.616 ***	0.516 ***	0.591 ***	0.513 ***	0.546 ***	0.583 ***	0.561 ***	0.545 ***	0.791 ***	0.939 ***	0.732 ***		0.534	0.627	0.681	0.566	0.745	0.467
OU (Our)	0.527 ***	0.471 ***	0.601 ***	0.443 ***	0.426 ***	0.516 ***	0.418 ***	0.327 ***	0.402 ***	0.377 ***	0.371 ***	0.481 ***	0.360 ***	0.372 ***	0.665 ***	0.820 ***	0.546 ***	0.710 ***		0.028	0.388	0.043	0.274	0.228

Table 3 *Continued*

Population	Elbe							Danube							Rhine						Maas		Weser	
	ZI	SR	WB	HB	MB	WE	ST	BL	WN	BI	WR	KO	RA	LE	WA	KA	ME	SC	OU	SU	AN	RU	LU	VB
SU	0.610	0.554	0.695	0.527	0.502	0.661	0.524	0.399	0.492	0.469	0.449	0.551	0.432	0.465	0.752	0.915	0.617	0.835	0.154		0.404	0.012	0.281	0.227
(Sauer)	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***					
AN	0.592	0.535	0.654	0.525	0.508	0.601	0.543	0.474	0.506	0.457	0.494	0.551	0.502	0.534	0.699	0.872	0.660	0.821	0.651	0.761		0.350	0.423	0.563
(Anlier)	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***				
RU	0.627	0.569	0.714	0.543	0.517	0.701	0.549	0.404	0.499	0.458	0.457	0.549	0.433	0.471	0.763	0.940	0.640	0.858	0.243	0.132	0.773		0.264	0.179
(Rulles)	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	**	***			
LU	0.320	0.269	0.421	0.237	0.205	0.236	0.201	0.175	0.206	0.254	0.180	0.246	0.219	0.242	0.535	0.697	0.313	0.628	0.394	0.482	0.541	0.496		0.434
(Lutter)	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***		
VB	0.473	0.452	0.599	0.406	0.396	0.396	0.373	0.232	0.367	0.294	0.292	0.380	0.324	0.287	0.691	0.936	0.530	0.734	0.422	0.619	0.732	0.669	0.381	
(Vogelsberg)	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	

of populations from different drainages is not evident from the NJ dendrogram. For instance, populations from today's northernmost Danube drainage (WN) and from the upstream Main/Rhine drainage (Metzlersreuther Bach, ME) both cluster closer to the geographical adjacent Elbe populations. Similarly, the BL population from the eastern part of the Bavarian forest clusters together with the geographically adjacent Danubian populations instead of grouping together with other Elbe populations. In the contact zone of Maas and Rhine drainages, the RU population from the Maas drainage clusters to the adjacent Rhine populations SU and OU. These results are supported by assignment tests (Table 4). An average of 79.4% (ranging from 38% to 100%) of the individuals was correctly assigned to its population of origin and a higher percentage of 93.0% (ranging from 65% to 100%) was correctly assigned to its drainage of origin at present. The lowest levels of correct assignment to the present-day drainage system mostly occurred in populations which are situated in the contact zones with adjacent drainages (e.g. ST, BL, WN, SU). The lowest levels of correct assignment to specific rivers within certain drainages were found for populations which once were or still are connected. For instance, in the interconnected Zinnbach–Wolfsbach–Südliche Regnitz system (see Fig. 1), out of 26 individuals analysed from Zinnbach (ZI), 58% is correctly assigned to its brook of origin, 23% is assigned to the Wolfsbach (WB) and 15% to the Südliche Regnitz (SR). In one case (WA), more than 50% of the individuals were assigned to an adjacent population.

Populations with 100% levels of correct assignment to their rivers of origin (KA, ME, SC, AN, LU, VB) can be considered to be genetically distinct and show long branches in the NJ dendrogram with highly supported bootstrap-values. In most cases, their uniqueness is supported by private alleles as well. However, there are also populations with private alleles (BL, SU), which neither yield high values in the assignment tests nor appear as clearly separate and well-supported branches in the dendrogram.

The heterozygosity contribution to differentiation (Fig. 2) reflects the above described results and shows that genetically variable populations from the Elbe and Danube drainage are usually those with low differentiation indices, whereas populations with a low genetic variability from Rhine, Maas and Weser catchment are those with the highest differentiation indices. The two populations from WA and KA show a remarkable genetic contribution.

Based on the results of the 2MOD program (Ciofi & Bruford 1999), the strong differentiation of the pearl mussel populations suggests a low level of gene flow between the extant populations. The relative likelihood of the model of gene flow–drift equilibrium vs. drift revealed a drift-model for the central European freshwater pearl mussel populations ($P = 1.0$).

Table 4 Assignment test for freshwater pearl mussel (*Margaritifera margaritifera* L.) populations based on the Bayesian method ('as it is' option) implemented in the GENECLASS 1.0.02 program (Piry & Cornuet 1999)

	Elbe								Danube								Rhine					Maas		Weser		
Population	ZI	SR	WB	HB	MB	WE	ST	BL	WN	BI	WR	KO	RA	LE	WA	KA	ME	SC	OU	SU	AN	RU	LU	VB	All
ZI (Zinnbach)	**15**	2	1					1																	19
SR (Südl. Regnitz)	4	**19**	1			1																			25
WB (Wolfsbach)	6	2	**22**					1																	31
HB (Höllbach)	1	2		**14**	2		1		2		1														23
MB (Mähringsbach)				3	**18**		1		1																23
WE (Weiße Elster)				1	3	**4**	2																		10
ST (Steinselb)				3			**9**		2																14
BL (Blanice)								**25**							1										26
WN (Waldnaab)				3	2		3	1	**20**		1	2													32
BI (Biberbach)								1	1	**23**			1												26
WR (Wolferstr. B)											**16**		2	2											20
KO (Kleine Ohe)								1			1	**28**	1												31
RA (Ranna)								1		1	1	1	**23**												27
LE (Leitenbach)								1		1		1	2	**22**											27
WA (Waldaist)											1				**9**										10
KA (Kamp)															14	**24**									38
ME (Metzlersr. B)				1		1											**26**								28
SC (Schondra)																		**20**							20
OU (Our)																			**20**	1					21
SU (Sauer)																			4	**16**		**4**			24
AN (Anlier)																					**26**				26
RU (Rulles)																			3	9		**21**			33
LU (Lutter)																							**19**		19
VB (Vogelsberg)								1																**4**	5
Sample size	26	25	24	25	25	6	16	33	26	25	21	32	29	24	24	24	26	20	27	26	26	25	19	4	558
Observed number assigned to sample site	15	19	22	14	18	4	9	25	20	23	16	28	23	22	9	24	26	20	20	16	26	21	19	4	443
Percent correctly assigned to sample site	58	76	92	56	72	67	56	76	77	92	76	88	79	92	38	100	100	100	74	62	100	84	100	100	79.4
Observed number assigned to main drainage of origin	26	25	24	21	23	5	13	27	21	25	20	32	29	24	23	24	26	20	24	17	26	21	19	4	519
Percent correctly assigned to drainage of origin	100	100	100	84	92	83	81	82	80	100	95	100	100	100	96	100	100	100	89	65	100	84	100	100	93.0

Discussion

Population structure

The results of the microsatellite analyses clearly reveal a high degree of population substructure among extant central European pearl mussel populations. They also show that diversity within pearl mussel populations differs strongly and only slightly correlates with census population size. Differences in genetic variation can generally be explained by (i) disequilibrium of mutation and selection connected with the evolutionary history of populations, and (ii) disequilibrium of drift and migration linked with the effects of fragmentation of populations and their demographic background. Detailed genetic analyses are required for the identification of priority populations for conservation with respect to their uniqueness in terms of genetic divergence from other populations and regarding their genetic diversity. Microsatellites with their high resolution are the markers of choice for these investigations of pearl mussel populations. The use of shell morphology characters can be deceptive when describing differentiation among mussel populations, as these characters largely depend upon environmental variables (e.g. Johnson 1970; Watters 1994). In fact, the use of ecophenotypic characters has led to a confusing number of contentious or uncertain taxa of lesser rank among pearl mussels and has produced confused or disputed taxonomies (Chesney & Oliver 1998) which can result in poor conservation strategies. However, in some cases morphologically atypical mussels (e.g. those from SC and RU) showed a strong genetic divergence to other populations. For the majority of populations, a link between genetic status and shell shape was not evident, underscoring the strong influence of environmental variables on these characters.

With respect to the taxonomic insufficiency and disputed taxonomy of ecophenotypes among freshwater pearl mussel populations, we use the term conservation unit (CU) as defined by Moritz (2002), Luck *et al.* (2003) and Manel *et al.* (2003) for a population or a group of populations important to be conserved. The conservation goals attributed to the concept of CUs for freshwater pearl mussel populations involve maintaining genetic diversity in the species, combining concepts of minimum viable populations (Soulé 1987; Nunney & Campell 1993), evolutionary significant units, ESUs (Moritz 1994; Crandall *et al.* 2000), and management units, MUs (Moritz 1994).

The genetic diversity and differentiation of pearl mussel populations found in this study can be explained by different factors, including colonization from different glacial refugia, postglacial recolonization and the generally complex colonization of new habitats as the result of the specificity between pearl mussel glochidia and their narrow spectrum of host fish vectors. Population structure is additionally influenced by the fact that the species reveals a specialization on clear and cold streams of the trout region with low levels of nutrients and lime, limiting the potential geographical distribution range. Moreover, anthropogenic factors like habitat alteration, water pollution effects and destructive pearl fishing have driven many populations to extinction or left small fragmented remnant populations. The current population structure of pearl mussel populations can thus be described as an anthropogenic fragmented metapopulation, showing stronger susceptibility to the loss of genetic variability and risk of extinction than other population structures. This explanation is also supported by the results of the model of gene flow–drift equilibrium vs. drift, which revealed predominant drift effects and by the fact that significant bottleneck effects were detected in many populations.

Additionally, our study shows that present-day population differentiation does not always match with present-day drainage systems, revealing the complex pattern of pre- and postglacial colonization in the contact zones of drainage systems. This effect can most likely be explained by historical changes in the flow direction of individual tributaries towards different drainages, postglacial effects and the temporal connections between different drainage systems at those times (for details, see Hantke 1993). In contrast to our results, allozyme data for the cold-adapted bullhead (*Cottus gobio*) showed a marked genetic differentiation across drainage basins in the contact zone of Elbe, Danube and Main/Rhine in northern Bavaria (Hänfling & Brandl 1998). These differences can be most likely explained by different dispersal and colonization patterns between bullhead and brown trout (*Salmo trutta*) as the host fish vector for pearl mussels. Data on the genetic structure of the much more dispersing brown trout would be more conclusive in this respect. Genetic studies of brown trout (e.g. Bernatchez 2001; Weiss *et al.* 2001), however, do not match with the distribution and sampling pattern of pearl mussels investigated in this study.

Distinct CUs for freshwater pearl mussel populations are not restricted to different drainages. Simultaneously, CUs are found within drainage systems. For instance, the Danubian drainage system is subdivided into three groups: a southern Danubian cluster of Austrian WA and KA populations, a cluster of central populations and the most upstream WN population, which groups with the Elbe populations.

The analyses of genetic diversity revealed significant differences both between drainages and between populations within drainage systems. Low levels of genetic diversity within certain populations can be the result of the fragmented metapopulation structure, implying founder effects and bottlenecks. According to IAM and TPM, recent bottlenecks were detected in populations from the drainages of Rhine, Elbe and Danube. The high number of monomorphic microsatellite loci in five other populations (KA,

SC, SU, AN, RU) prevented them from being tested for excess of heterozygotes with the BOTTLENECK approach. The high number of monomorphic loci together with the high F values suggest that bottlenecks may also have had predominant effects in these populations. The fact that all Danubian populations in Bavaria showed heterozygote excess could be explained by recent anthropogenic influences, as all of the populations in this area were intensively exploited after the regal right to harvest pearl mussels was abolished in this region in the year 1874 (Meißner 1912). Furthermore, the species' extraordinary life cycle suggests a higher likelihood for the effects of small populations such as inbreeding and drift. The ability of female pearl mussels to switch to hermaphrodites at low densities of males and the enormous reproduction potential of single individuals (Bauer 1987) can to some extent explain the comparatively low measures of genetic diversity accomplished by high census population sizes. However, the species' reproduction strategy suggests that pearl mussels may be less susceptible to inbreeding depression than other species. In fact, a viable and well reproducing population from Portugal (Geist, unpublished communication) shows very low levels of genetic variability. However, the only two populations included in this study which still show high levels of reproduction (BL and LU) are among those with the highest intrapopulation diversity indices. Within interconnected river systems with extant pearl mussel populations in different tributaries (e.g. Zinnbach–Wolfsbach–Südliche Regnitz), genetic diversity was usually observed to be lowest in the smallest headwater streams, in which recent population bottlenecks were detected with higher probability (e.g. Zinnbach). This observation could be explained by factors of environmental stochasticity, like the higher risk for small headwater tributaries to fall dry during summer or freeze completely during winter. In this case, extinction and recolonization led to the observed lower indices of genetic variability.

Conservation and management implications

When implying sustainable conservation management and recovery strategies for freshwater pearl mussel populations, the loss of genetic diversity should be minimized by retaining the CUs. First, it is the distinctiveness and differentiation of a population in comparison to other extant populations in terms of its allelic composition. Populations which are characterized by an independent evolutionary history, as indicated by private alleles, high F_{ST} values, long branches with high bootstrap support in the phenogram, and a low percentage of misclassification in the assignment test, can be considered as separate conservation units (CUs), as in the case of Lutter (LU), Vogelsberg (VB), Schondra (SC), Metzlersreuther Bach (ME), Anlier (AN) and Our (OU). Within the Danubian drainage, three different CUs can be defined: A downstream group comprising Waldaist (WA) and Kamp (KA), a central Danubian group (Leitenbach, LE; Kleine Ohe, KO; Ranna, RA; Biberbach, BI; Wolfertsrieder Bach, WR) and the northernmost Waldnaab (WN) unit. Elbe populations can be subdivided into two CUs, a Northern Bavarian group (Steinselb, ST; Mähringsbach, MB; Höllbach, HB; Weiße Elster, WE; Südliche Regnitz, SR; Zinnbach, ZI; Wolfsbach, WB) and the separate Czech Blanice (BL) population. This also implies that no stocking attempts with mussels or glochidia from other distinct CUs should be carried out within these populations as long as individuals from the original populations are still present. The maintenance of several isolated populations can actually increase overall genetic diversity, because allelic differences can be preserved as a result of local adaptation to different habitats.

Adaptive differences between CUs as a result of different natural selection pressures may occur, despite the fact that differentiation between populations is additionally enhanced by drift effects. Mixing with other populations could thus result in outbreeding depression, i.e. the reduction in fitness caused by the breakdown of co-adapted gene complexes (Templeton 1986). There were several unsuccessful attempts in Germany to found new populations by translocating mussels from one river to other rivers (Scherf 1980). Other studies revealed a one-year survival rate of only 50% for inter-river transfers of pearl mussels in Finland (Valovirta 1990). Despite the fact that other reasons cannot be ruled out in these cases, both observations indicate local adaption of pearl mussels to specific habitats and suggest that CUs are important to be recognized in pearl mussel conservation. With exception of WA, the results of the assignment test can be well explained by natural evolutionary (colonization/demography) patterns. It has to be mentioned, however, that possible historical stocking activities with mussels from populations that are nowadays extinct could not be detected in this study.

Despite the recommendation to manage distinct CUs separately, it is essential to minimize the loss of genetic diversity within populations, as loss of genetic heterozygosity can have deleterious effect on population fitness (e.g. Reed & Frankham 2003). Conservation management and recovery strategies such as semiartificial breeding and culturing techniques, have to balance between maintenance of genetic divergence and diversity. A drift–migration equilibrium, as it can be achieved by rotation crossing (Kimura & Crow 1963), would ideally meet these criteria. It has to be considered, however, that freshwater pearl mussels, with the ability of females to switch to hermaphrodites at low population densities (Bauer 1987) are probably better adapted to inbreeding effects than other animal species.

For supportive breeding in interconnected river systems, like those of ZI, WB and SR (see Fig. 1), it would be sufficient to collect glochidia from Südliche Regnitz and subsequently release them to the upstream tributaries ZI and WB, because

genetic variability is highest in the most downstream SR and no other alleles are found in the upstream tributaries of ZI and WB.

Management guidelines can be recommended, according to a classification of extant pearl mussel populations into four separate categories: large populations with high genetic diversity, small populations with high diversity, large populations with low diversity and small populations with low diversity.

In general, large populations with high diversity indices such as BL seem to have been stable consistently or fluctuated at high population densities with high levels of intrapopulation gene flow, low levels of hermaphrodism and no recent bottlenecks. It is likely as well that such populations have had high densities of host fish and large areas of suitable substrate for the development of juveniles, allowing a diversity of offspring from different parent mussels to grow up naturally and continuously. From the conservation point of view, they are probably less susceptible to be driven to extinction than other populations and habitat protection can be considered to be the most important conservation tool.

High diversity indices in small populations like ST can probably be explained by historically intact and large populations that have faced a strong recent decrease because of anthropogenic deterministic effects of habitat destruction, water pollution or over-exploitation that are not linked to genetic selection. Bottleneck effects, however, would probably be detected in the offspring of these populations and can be avoided by applying breeding strategies on a genetic basis. Such populations deserve high priority in conservation and should be recovered as quickly as possible, in order to avoid the effects of genetic stochasticity on small populations. In areas, in which genetically closely related populations from the same CU are still available (e.g. central Danubian CU), gene flow between these populations may be advantageous.

In contrast, the genetic status of large populations with low diversity levels and low effective population sizes like KA can most likely be explained by colonization with few founder individuals or pronounced population bottlenecks in the past, followed by a subsequent recovery. Management strategies in such populations should try to maintain diversity by selecting genetically different parental individuals.

Small populations with low levels of diversity (like SC or RU) seem to have been isolated relict populations for quite a long time, probably characterized by a continuous decline in genetic diversity over a long period. Special concern should be attributed to avoid further loss of genetic diversity in these populations when implying artificial breeding and culturing techniques.

Conclusions

Our data show that detailed genetic analyses are mandatory for selecting priority populations for conservation because: (i) genetic differentiation does not always correlate with geographical distance, i.e. populations with private alleles and high F_{ST} values can occur even within drainage systems, and (ii) actual census population sizes only weakly correlate with F values ($r^2 = 0.05$ and $P = 0.288$), i.e. present-day large populations are not necessarily those with high diversity levels and effective population sizes. Thus, from a genetic point of view, a sound and effective management strategy cannot only focus on the protection and the support of comparatively large remaining populations from geographically distinct areas.

The issue of defining conservation and management strategies for freshwater pearl mussel populations clearly illustrates the challenges involved in conservation of endangered species, and is closely connected with the problem of choosing a single large refuge rather than several small refuges in island biography, the so-called SLOSS controversy (Simberloff & Abele 1982). Sustainable management and recovery of pearl mussel populations can benefit from a combined approach, integrating applications of ecological science with the selection of priority populations based on genetic criteria for differentiation and diversity.

Acknowledgements

This work was conducted as part of a research project on 'Genetics and Ecology of European Pearl Mussel Populations', which is funded by 'Landesfischereiverband Bayern' and 'Bayerischer Naturschutzfonds'. We would like to thank R. Altmüller, M. Lange, St. Bocca, St. Terren, G. Motte, K.-O. Nagel, J. Hruska, T. Ofenböck, J. Moser, G. Lehner-Meier, G. Nowak, Ch. Schmidt, St. Schmidt, G. Wenz and all authorities involved for their assistance during sample collection and for providing special licenses to work with endangered pearl mussels. We are grateful to W. Schröder and O. Rottmann for their continued support and to B. Hänfling for useful comments on the manuscript.

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This work is part of Jürgen Geist's PhD project on conservation genetics and ecology of freshwater pearl mussels in Europe. J. Geist's research interests focus on the conservation biology of endangered freshwater species, combining molecular aspects of conservation genetics with ecological approaches of habitat quality evaluation and restoration. Ralph Kuehn runs the conservation genetics laboratory at the Center of Life Science Weihenstephan at Technische Universität München. His scientific orientation concentrates on the combination of micro- and macro-scaled studies of endangered species in the context of Molecular Ecology and Conservation Genetics.
