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Comparison of microsatellite variability in wild and cultured tench (*Tinca tinca*)

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Abstract

The tench is a cyprinid fish species of increasing interest for European pond aquaculture. However, only little is known on the population genetic structure of this species; the few studies conducted so far were based on allozyme polymorphisms. In the present study, tench specific microsatellites were used for a genetic comparison of two wild populations and four cultured strains from Germany and Czech Republic. Seven out of nine loci analysed were polymorphic with a total number of alleles ranging from 2 (locus *MTT-2*) to 19 (locus *MTT-9*). Wild populations showed a higher allelic richness than the cultured strains (3.73 *vs*. 2.43) but this difference was still not significant ($P=0.062$). Observed heterozygosity (0.370 *vs*. 0.366) and gene diversity (0.403 *vs*. 0.343) were globally similar in wild and cultured tench. In contrast to the variability within populations, genetic differentiation was significant between all of them as estimated by F_{ST} values from pairwise comparisons. The differentiation of the two wild populations (F_{ST} of 0.024) was lower than the average differentiation of the four cultured strains (mean F_{ST} of 0.159) but again this difference was not significant ($P=0.143$). The present results indicate a tendency towards a reduction in variability within and an increase in differentiation between cultured strains in comparison to wild populations. To verify this, more populations have to be analysed. However, it is already evident that wild tench populations should be protected since they represent valuable genetic resources.

Keywords: Genetic variability; Microsatellites; Tench; *Tinca tinca*

1. Introduction

The traditional Central and Eastern European freshwater pond fish culture is clearly dominated by the common carp (*Cyprinus carpio* L.). Changes in economic conditions and partly also in market preferences, however, resulted in an increasing interest of fish farmers to diversify their production in terms of species, in particular in the former socialist countries after the political transitions that took place there in the 1990s. Several possible candidate species had been identified, e.g. European catfish (*Silurus glanis* L.), sturgeons (*Huso* and *Acipenser* sp.) and their interspecific hybrids, pike perch (*Sander lucioperca* (L.)) and ornamental varieties of various fishes. A special attention was paid to the tench (*Tinca tinca* L.) since it has already been reared in ponds as so-called secondary or by-fish for hundreds of years (Steffens, 1995).

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One of the major constraints of tench farming is its slow growth in comparison to many other fish species (Steffens, 1995). The potential for genetic improvement of tench has been reviewed by Flajšhans et al. (1998): A breeding programme has been established in the Czech Republic focusing on the creation of strains with higher homozygosity and crossbreeding to utilize heterosis effects as it has been described by Kvasnička and Linhart (1990). Increased homozygosity was achieved by full-sib mating or by meiotic gynogenesis. Experiments on induced triploidy indicated a possible production enhancement by a monosex culture of triploid tench females. On the other hand, basic genetic studies on the variability and differentiation of tench populations are still rare, restricted to hatchery reared stocks from the Czech Republic (Šlechtova et al., 1995) and one wild population from a German lake (Kohlmann and Kersten, 1998), and were based on allozyme polymorphisms. These markers showed moderate and rather similar levels of variability within populations: 1 to 1.5 *vs*. 1.3 alleles per locus; 0 to 37% *vs*. 30.4% of polymorphic loci; mean observed heterozygosities of 0 to 0.119 *vs*. 0.081 in the cultured stocks and in the wild population, respectively. Only recently tench specific microsatellite loci had been isolated (Kohlmann and Kersten, 2006). As could be expected, their variability was much higher than that of the allozyme loci making them a more suitable tool for population genetic studies in tench.

Many studies on fish demonstrated a reduced genetic variability of cultured stocks in comparison to wild populations, e.g. in masu salmon, *Oncorhynchus masou* Brevoort (Nakajima et al., 1986), rainbow trout, *Oncorhynchus mykiss* Walbaum (Paaver, 1986), Atlantic salmon, *Salmo salar* L. (Staahl, 1983; Verspoor, 1988), brown trout, *Salmo trutta* L. (Vuorinen, 1984) and common carp (Kohlmann et al., 2003, 2005). Therefore, the aim of the present study was to evaluate if similar processes already took place in tench using microsatellite loci as genetic markers. The anticipated results will have implications not only on the further culture and breeding of tench but also for the conservation of the genetic resources of this species.

2. Material and methods

Tench were collected from two wild populations (Lake Döllnsee and Lake Felchowsee, Germany) and four cultured strains (Königswartha, Germany, and Tabor, Marianske Lazne and Vodnany, Czech Republic). Sample sizes ranged from 40 to 50 individuals with the exception of the Lake Döllnsee population from which only 19 fish were directly caught in the wild. The individuals from this lake had already been examined for allozyme polymorphisms (Kohlmann and Kersten, 1998) and had also been used to study the variability of newly isolated tench specific microsatellite loci (Kohlmann and Kersten, 2006). The cultured strains were named after the geographical locations they were originally reared in (for details on Czech strains see Flajšhans et al., 1999).

Genomic DNA was isolated from muscle samples using the E.Z.N.A. Tissue DNA Kit II (Peqlab Biotechnologie). The nine tench specific microsatellite loci were amplified by PCR. Primer sequences can be found in Kohlmann and Kersten (2006) or in GenBank under accession numbers DQ080084 to DQ080092. Each PCR reaction mix was composed of 1.5 μl of 10 × PCR buffer with $(NH_4)_2SO_4$ (MBI-Fermentas), 1.2 μl of 25 mM $MgCl_2$, 1.2 μl of 1.25 mM dNTPs, 0.3 μl of each primer (10 pmol/μl), 3 μl genomic DNA, 0.1 μl of *Taq* DNA-polymerase (5 units/μl; MBI-Fermentas) and sterile water up to a final volume of 15 μl. The forward primer of each pair was labelled with one of the WellRed fluorescence dyes D2-PA, D3-PA, and D4-PA, respectively (Proligo). For all loci the PCR reaction amplification consisted of an initial denaturation at 95°C for 5min, followed by 5 cycles consisting of denaturation at 95°C for 1min, annealing at 55°C for 1min, extension at 72°C for 1min and another 35 cycles consisting of denaturation at 90°C for 1min, annealing at 55°C for 1min, extension at 72°C for 1min. A final extension step at 72°C lasted for 7min.

Allele sizes were determined on a CEQ 8000 (Beckman Coulter) using the 400bp internal size standard. Alleles were designated according to their size in base pairs. Genotypes were recorded and used as input data for the GENEPOP software package (Raymond and Rousset, 1995) to calculate allele and genotype frequencies, observed and expected heterozygosities and to test for deviations from Hardy–Weinberg equilibrium and for the significance of heterozygote deficiency and excess, respectively (probability test: estimation of exact *P*-values by the Markov chain method). Genetic variability within groups of populations (allelic richness, observed heterozygosity, gene diversity and F_{IS} values) as well as genetic differentiation between populations (F_{ST} values) was estimated using the FSTAT software (Goudet, 2002). In all cases with multiple tests, significance levels were adjusted using the sequential Bonferroni correction (Rice, 1989).

3. Results and discussion

Seven out of the nine microsatellite loci analysed were polymorphic with a total number of 49 alleles being recorded across all polymorphic loci (Table 1). The number of distinct alleles at polymorphic loci displayed large variation, ranging from 2 (locus *MTT-2*) to 19 (locus *MTT-9*). Interestingly, locus *MTT-3* was variable (three alleles) only in the cultured tench from Königswartha. With the exception of the cultured strains from Königswartha (two alleles at locus *MTT-3*) and Marianske Lazne (one allele at locus *MTT-6*), private alleles could only be found in the two German wild populations. Most of them (seven alleles) occurred in the Lake Felchowsee population at the most variable locus *MTT-9*. The average number of alleles per locus was the lowest in the cultured Vodnany strain (2.57) and the highest in wild tench from Lake Felchowsee (5.86).

Genetic variability parameters – allelic richness (based on a minimum sample size of 17 individuals from the wild Döllnsee population), observed heterozygosity and gene diversity (i.e. expected heterozygosity) – were higher in wild populations than in cultured strains, but the differences between the two groups were statistically not significant (Table 2). However, is has to be noticed that the locus with the highest total number of alleles (*MTT-9*) showed the largest difference in average number of

Table 1
Variability of six tench populations at seven polymorphic microsatellite loci (n = number of fish; A = number of alleles; A_P = number of private alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; P_{HW} = exact P-value of Hardy–Weinberg probability test; n.a. = not applicable)

Locus	Parameter	Döllnsee	Felchowsee	Königswartha	Tabor	Marianske Lazne	Vodnany	Number of distinct alleles
MTT-1	n	19	47	50	40	42	50	7
	A	5	7	4	4	4	4	
	A_P	–	2	–	–	–	–	
	H_O	0.632	0.872	0.740	0.750	0.333	0.540	
	H_E	0.740	0.788	0.663	0.708	0.407	0.427	
	P_{HW}	0.043	0.455	0.302	0.744	0.111	0.004	
MTT-2	n	19	49	50	40	42	50	2
	A	2	2	2	2	2	2	
	A_P	–	–	–	–	–	–	
	H_O	0.579	0.327	0.220	0.350	0.452	0.560	
	H_E	0.462	0.374	0.198	0.380	0.441	0.476	
	P_{HW}	0.345	0.442	1.000	0.678	1.000	0.241	
MTT-3	n	19	49	50	40	42	50	3
	A	1	1	3	1	1	1	
	A_P	–	–	2	–	–	–	
	H_O	–	–	0.560	–	–	–	
	H_E	–	–	0.446	–	–	–	
	P_{HW}	–	–	0.075	–	–	–	
MTT-5	n	19	49	50	40	42	50	4
	A	4	4	4	2	3	3	
	A_P	–	–	–	–	–	–	
	H_O	0.474	0.571	0.960	0.550	0.667	0.500	
	H_E	0.661	0.645	0.755	0.506	0.605	0.516	
	P_{HW}	0.089	0.217	0.000	0.752	0.176	1.000	
MTT-6	n	18	49	50	40	42	50	9
	A	6	5	5	5	4	3	
	A_P	1	–	–	–	1	–	
	H_O	0.722	0.571	0.840	0.825	0.690	0.620	
	H_E	0.624	0.708	0.615	0.744	0.626	0.491	
	P_{HW}	0.385	0.190	0.003	0.835	0.553	0.091	
MTT-8	n	17	49	50	40	42	50	5
	A	3	3	2	2	3	2	
	A_P	1	–	–	–	–	–	
	H_O	0.118	0.102	0.240	0.300	0.024	0.000	
	H_E	0.169	0.240	0.213	0.292	0.313	0.272	
	P_{HW}	0.091	0.000	1.000	1.000	0.000	0.000	
MTT-9	n	18	49	50	40	42	50	19
	A	9	19	5	4	4	3	
	A_P	–	7	–	–	–	–	
	H_O	0.889	0.857	0.720	0.700	0.786	0.240	
	H_E	0.859	0.901	0.666	0.616	0.697	0.305	
	P_{HW}	0.542	0.878	0.002	0.739	0.000	0.010	
	Average number of alleles per locus	4.29	5.86	3.57	2.86	3.00	2.57	
	Average H_O	0.379	0.367	0.476	0.386	0.328	0.273	
	Average H_E	0.391	0.406	0.395	0.361	0.343	0.276	
	P_{HW}	0.043	0.008	0.000	0.996	0.000	0.000	
	Average F_{IS}	0.030	0.098	−0.206	−0.071	0.045	0.010	
	P-value for F_{IS}	n.a.	0.000	0.000	n.a.	0.006	0.004	

Table 2
Genetic variability within and differentiation between wild populations and cultured strains of tench

	Allelic richness	Observed heterozygosity	Gene diversity	F_{IS} value	F_{ST} value
Wild	3.73	0.370	0.403	0.082	0.024
Cultured	2.43	0.366	0.343	−0.069	0.159
Two-sided *P*-values	0.062	1.000	0.348	0.275	0.143

alleles between the two groups: 9 and 19 in the two wild populations *vs*. 3 to 5 in the four cultured strains.

Results of the Hardy–Weinberg probability tests revealed no regular patterns for significant/non-significant deviations from equilibrium at single loci (Table 1). However, it is remarkable that locus *MTT-8* showed highly significant deficiencies of heterozygotes in half of the studied populations. This observation might suggest the presence of null allele(s) at this locus leading to a false recording of null allele heterozygotes as homozygotes for the variant allele. On the other hand, none of the tench samples from these populations failed to amplify this locus what would have been an indication for null allele homozygotes. At the population level only the cultured Tabor strain was found to be in equilibrium. The low sample size of 19 individuals might have contributed to the significant deviation of the wild Lake Döllnsee population from equilibrium but its P_{HW}-value (0.043) was close to the threshold of 0.05 and became non-significant after sequential Bonferroni corrections (initial *P*-value of 0.05 divided by 9 tests = 0.0055). The highly significant deviation from equilibrium in the wild tench from Lake Felchowsee was caused by a highly significant deficiency of heterozygotes at only one locus: *MTT-8*. A possible explanation for the deviations observed at this locus has already been given above. The two cultured strains from Marianske Lazne and Vodnany showed both a highly significant overall deficiency of heterozygotes (the *P*-values for F_{IS} were 0.006 and 0.004, respectively), at least partly due to deviations at locus *MTT-8* again. However, after sequential Bonferroni corrections only the F_{IS} value for the Vodnany strain remained significant but was very close to the adjusted nominal level for multiple comparisons (0.0055). In connection with the tendency of a reduced intrapopulational genetic variability, this might be an indication for low to medium levels of inbreeding. In contrast, the cultured strain from Königswartha was the only one that expressed a highly significant overall excess of heterozygotes (Table 1) that remained highly significant even after sequential Bonferroni corrections. Unfortunately, no information on the breeding history of this strain is available — mixing with other strains and possible crossbreeding as a source of heterozygote excess could therefore not be excluded.

In contrast to the non-significant differences in the genetic diversity observed within samples, genetic differentiation was significant ($P<0.05$) for all pairwise comparisons of populations and strains (Table 3). All F_{ST} values remained significant after sequential Bonferroni corrections (initial *P*-value of 0.05 divided by 15 tests = 0.0033). Although average genetic differentiation between the two wild populations (F_{ST} of 0.024) was lower than average differentiation between the cultured strains (mean F_{ST} of 0.159), the difference between the two groups was not significant (Table 2).

The present results indicate a tendency towards a reduction in variability within and an increase in differentiation between cultured tench strains in comparison to wild populations. The decline of genetic variability within the cultured tench strains – so far mainly a loss of (rare) alleles rather than a reduced heterozygosity – might be caused by a relatively low number of breeders used to maintain these strains. Such situations are typical for fish culture due to the generally high fecundity of females. In the long term this may lead to measurable inbreeding depressions such as reduced vitality and growth rate. Therefore, inbreeding should be minimized by increasing the effective population sizes. According to a FAO/UNEP (1981) recommendation, the increase of the inbreeding coefficient should not exceed 1% per generation. This requires an effective population size of at least 50 individuals (25 males and 25 females). The increase of genetic differentiation between the cultured tench strains might be attributed to two major factors: (1) founder effects at the time when the cultured strains were established and (2) genetic drift during the culture process. In addition, genetic adaptation to local culture conditions and artificial selection could also be involved. The resulting genetically differentiated strains could be a valuable basis for future crossbreeding programmes. Preliminary data indicate that tench strain hybrids may not only show superior growth but also a higher product quality than pure strains (Wedekind et al., 2003).

On the other hand, it cannot be completely excluded that the present results on genetic differences between wild and cultured tench are confounded to some extent with a yet

Table 3
F_{ST} estimates for pairwise comparisons of tench populations

	Döllnsee	Felchowsee	Königswartha	Tabor	Marianske Lazne	Vodnany
Döllnsee	–					
Felchowsee	0.0235	–				
Königswartha	0.0912	0.0828	–			
Tabor	0.0929	0.0636	0.1173	–		
Marianske Lazne	0.0734	0.0804	0.1738	0.1257	–	
Vodnany	0.1694	0.1408	0.2439	0.1116	0.1366	–

All values are statistically significant at the 5% level and after sequential Bonferroni corrections.

unknown geographical structuring of populations: the wild tench originated in Germany and all but one cultured strains came from Czech Republic. Therefore, the comparison of the two groups should be considered cautiously and preliminarily. Our future genetic research on tench will be directed to analyse a larger number of natural populations more widely distributed as well as a larger number of cultured stocks in order to verify the trends of genetic changes observed in the present study. However, it is already evident that wild tench populations should be protected since they represent valuable genetic resources.

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