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RESEARCH PAPER

Genetic variability and differentiation of wild and cultured tench populations inferred from microsatellite loci

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Abstract Nine species-specific microsatellites were used to characterize 792 tench, *Tinca tinca* (L.), from 21 wild and cultured populations. Seven loci were polymorphic expressing four to 22 alleles. A Spanish cultured strain was homozygous at all loci for all individuals studied. Low variability was also observed in a wild population from Sapanca Lake, Turkey and a Chinese cultured strain. In contrast, the highest variabilities were found in wild tench from lake Felchowsee (average number of alleles), and the cultured strain from Königswartha (average heterozygosity), both from Germany. Genetic differentiation between populations was moderate to high. The smallest genetic distances were found between the geographically most distant populations. A Neighbor-Joining tree showed only two major clades consisting of 4 and 17 populations, respectively. Within the smaller clade the Turkish wild and Spanish and Chinese cultured tench formed a sub-cluster with 100% bootstrap support. Possible reasons for the latter unexpected grouping are discussed.

Keywords Microsatellites · Population genetics · Tench · *Tinca tinca*

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Introduction

Despite of the still growing interest in biology and culture of the tench, *Tinca tinca* (L.), only little is known on the genetic structure of its wild populations and cultured strains. The few studies published so far were based on allozyme polymorphisms. These markers showed moderate and rather similar levels of variability within populations: 1–1.5 versus 1.3 alleles per locus; 0–37% versus 30.4% of polymorphic loci; mean observed heterozygosities of 0–0.119 versus 0.081 in Czech cultured stocks (Šlechtová et al. 1995) and in a German wild population (Kohlmann and Kersten 1998), respectively. Nowadays, microsatellites are the preferred markers in population genetic research because of their high variability, co-dominant mode of inheritance and rapid evolution. Tong et al.

(2005) could demonstrate successful cross-species amplification of the microsatellite locus *MFW1* originally isolated from the common carp, *Cyprinus carpio* L., by Crooijmans et al. (1997) in several other cyprinid species. However, they reported only one allele in tench making this locus useless for the species. The first original, tench specific microsatellite loci (*MTT-1* to *MTT-9*) had been isolated by Kohlmann and Kersten (2006). Three of these loci were found to be monomorphic, and the remaining six loci segregated for two to nine alleles in a wild test population. The observed heterozygosities at polymorphic loci were high (0.500–0.959) with only one exception (locus *MTT-8*: 0.167). Thus, as expected, the variability of tench microsatellites was higher than that of the allozymes. Subsequently, these new microsatellites were applied to compare two wild populations and four cultured tench strains from Germany and Czech Republic (Kohlmann et al. 2007). The results of that study indicated a tendency towards a reduction in genetic variability within and an increase in differentiation between cultured strains in comparison to wild populations.

In order to get a more comprehensive picture on the general genetic structure of tench both the number of populations/strains sampled and the geographic range of their origin were increased for the present study covering now three wild populations and 17 cultured strains from eight European countries as well as one cultured strain from East Asia (China).

Materials and methods

A total of 792 tench individuals were collected from 21 wild and cultured populations including golden and blue colour varieties (Table 1). Two out of three wild populations and four out of 18 cultured strains were already analysed by Kohlmann et al. (2007), and are indicated in Table 1. Sample sizes ranged from 14 to 16 in two Polish cultured strains and the two colour mutants up to 40–50 in most of the other populations. Generally, the samples were named after the geographical locations they were originally reared in or caught in the wild. For details on Czech strains see Kvasnička and Linhart (1990) and Flajšhans et al. (1999). The origin and colour inheritance of golden and blue tench were described by Kvasnička et al. (1998).

Genomic DNA was isolated from muscle, fin or blood samples using the peqGOLD Tissue DNA Mini Kit (Peqlab Biotechnologie). The nine tench specific microsatellite loci were amplified by PCR on a Mastercycler gradient apparatus (Eppendorf). Primer sequences can be found in Table 2 or in GenBank under accession numbers DQ080084 to DQ080092. Each PCR reaction mix was composed of 1.5 mm^3 of 10× PCR buffer with $(NH_4)_2SO_4$ (MBI-Fermentas), 1.2 mm^3 of 25 mM $MgCl_2$, 1.2 mm^3 of 1.25 mM dNTPs, 0.3 mm^3 of each primer (10 pmol/mm^3), 3 mm^3 genomic DNA, 0.1 mm^3 of *Taq* DNA-polymerase (5 units/mm^3; MBI-Fermentas) and sterile water up to a final volume of 15 mm^3. The forward primer of each pair was labelled with one of the WellRed fluorescence dyes D2-PA, D3-PA, and D4-PA, respectively (Sigma-Proligo). For all loci the PCR reaction amplification consisted of an initial denaturation at 95°C for 5 min, followed by 5 cycles consisting of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min and another 35 cycles consisting of denaturation at 90°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min. A final extension step at 72°C lasted for 7 min.

Microsatellite genotypes were recorded using the CEQ 8000 Genetic Analysis System, Fragment Analysis module (Beckman Coulter), and examined with MICROCHECKER (van Oosterhout et al. 2004). The GENEPOP software package (Raymond and Rousset 1995) was used to calculate allele numbers, observed and expected heterozygosities and to test for deviations from Hardy–Weinberg expectations as well as for deficiency or excess of heterozygotes (probability test: estimation of exact *P*-values by the Markov chain method). The number of private alleles was recorded at each locus and for each population. Genetic differentiation between populations was evaluated by calculating pairwise estimates of F_{ST} values and testing their significance by bootstrapping analysis (1,000 replicates) using the FSTAT software (Goudet 2002). FSTAT was also used to calculate F_{IS} values for each population. In all cases with multiple tests, significance levels were adjusted using the sequential Bonferroni correction (Rice 1989).

In order to examine the genetic relationships among populations a matrix of pairwise D_A distances (Nei et al. 1983) was calculated with bootstrapping (1,000 replicates) using the MSA program (Dieringer

Table 1 Description of the 21 tench populations examined for variation at nine microsatellite loci

Population label	Clear name	Number of fish	Remarks
Doel[a]	Döllnsee	19	German wild population
Felc[a]	Felchowsee	49	German wild population
Kowa[a]	Königswartha	50	German cultured strain
Ta98[a]	Tabor	40	Czech cultured strain, year class 1998
ML98[a]	Marianske Lazne	42	Czech cultured strain, year class 1998
Vo98[a]	Vodnany	50	Czech cultured strain, year class 1998
Vo96	Vodnany	50	Czech cultured strain, year class 1996
VoHy	Vodnany hybrid	50	Czech hybrid strain
VeMe	Velke Mezirici	50	Czech cultured strain
Hlub	Hluboka	50	Czech cultured strain
Gold	Golden	15	Colour variety developed in Vodnany
Blue	Blue	15	Colour variety developed in Vodnany
Ital	Italy	30	Ceresole d'Alba, cultured strain, natural reproduction
Bada	Badajoz	50	Spanish cultured strain collected at Las Vegas del Guadiana fish farm
Chin	China	31	Chinese cultured strain collected in Wuhan originating from few broodfish from northwest China
Roma	Romania	50	Romanian cultured strain collected at Vodnany live gene bank
Hung	Hungary	41	Hungarian cultured strain collected at Vodnany live gene bank
Turk	Turkey	50	Turkish wild population caught in Sapanca Lake
Poli	Poliwoda	14	Polish cultured strain
Szcz	Szczodre	16	Polish cultured strain
NoWi	Nowa Wieś	30	Polish cultured strain

[a] Populations already analysed by Kohlmann et al. (2007)

and Schlötterer 2003). The D_A distance measure was chosen because it is independent of the mutation models (Nei 1987) and superior to other distance measures in correct tree topology construction using microsatellites (Takezaki and Nei 1996). The resulting set of 1,000 distance matrices was then taken to run the NEIGHBOR and CONSENSE modules of the PHYLIP version 3.5c software package (Felsenstein 1993) in order to construct a consensus Neighbor-Joining tree. The publication-ready Neighbor-Joining tree was plotted using the MEGA program (Kumar et al. 2004). Finally, the interpopulational dispersal of individuals was assessed by an assignment test (GeneClass software; Cornuet et al. 1999) using the Likelihood and Bayesian method and algorithm.

Results

All of the nine microsatellite loci could be amplified successfully. In total, 66 alleles were recorded across loci with 20 of them being private alleles (Table 2). Thus, the mean number of alleles per locus was 7.33 at the species level. The number of distinct alleles showed large variation among loci. Two loci (*MTT-4* and *MTT-7*) were monomorphic in all tench individuals examined and were therefore excluded from all variability parameter calculations. The remaining seven polymorphic loci expressed four (*MTT-5*) to 22 (*MTT-9*) alleles. Evaluation of genotypes with the MICROCHECKER software indicated a possible presence of null allele(s) at locus *MTT-8* in 11 populations (Table 3).

Surprisingly, the Spanish cultured tench strain collected at the fish farm Las Vegas del Guadiana near Badajoz was homozygous at all loci for all 50 individuals analysed (Table 3). Low within population variability was also detected in the Turkish wild population caught in Sapanca Lake (1.29 alleles per locus; observed heterozygosity of 0.034) and in the Chinese cultured strain collected in Wuhan (1.71 alleles per locus; observed heterozygosity of 0.074).

Table 2 Primer sequences (F = forward; R = reverse), total number and number of private alleles, and size range of alleles observed at nine microsatellite loci in 21 tench populations

Locus	Primer sequences (5′ > 3′)	Total number of alleles	Number of private alleles	Size range (bp) of alleles
MTT-1	F: GTCCTCGCAATGCAAGAAAT	7	1	165–177
	R: TTGGCTCATATTGGGTGTGA			
MTT-2	F: CTGGTCTCCTCCTTGTGCTC	5	2	206–240
	R: TGGGTGAAGGATTGGTTGTT			
MTT-3	F: CCAGCAGAGCCCTACACTTC	5	2	148–164
	R: AGGACGTGACCATCAACACA			
MTT-4	F: TTAAAACCGCCACACTTTCC	1	–	211
	R: ACGTGCGGCTGTGAGATTAT			
MTT-5	F: GGGAGCCAGTTCACACTCAT	4	–	207–215
	R: GACATGAAAACGGTGCTGTG			
MTT-6	F: TGTGTGAGGTGGCACAGAAT	11	4	152–192
	R: ATGTGAGCAATGGCTGTGAG			
MTT-7	F: ACCTCGCCATGTATGCTTTT	1	–	213
	R: GTTGACCTGTGCATGCATTT			
MTT-8	F: GAAATGTCCCCACAAACCAC	10	6	178–236
	R: GACACCGCTATCACCATCAG			
MTT-9	F: CAATCTGGTGGAAGTGAGCA	22	5	124–182
	R: ACGCGTCAGTGACAGAGAGA			
$\sum$		66	20	

The remaining more variable populations expressed average numbers of alleles per locus ranging from 2.43 (Czech cultured strain from Vodnany, year class 1996) to 5.86 (German wild population caught in lake Felchowsee), and observed heterozygosities ranging from 0.351 (Czech cultured strain from Vodnany, year class 1998) to 0.611 (German cultured strain from Königswartha). However, the high heterozygosity of the Königswartha strain was at least partly due to the fact that these tench were among the few populations that showed variation at locus *MTT-3* (three alleles). The majority of cultured strains with a monomorphic locus *MTT-3* displayed observed heterozygosities up to 0.496 (Czech cultured strain from Tabor) and 0.504 (Czech cultured hybrid strain from Vodnany). At the population level, the number of private alleles ranged from zero to four (Table 3).

Hardy–Weinberg equilibrium tests revealed significant deviations ($P < 0.05$) in 15 populations mainly due to possible null allele(s) at locus *MTT-8* (null allele heterozygotes would be recorded as homozygotes for the variant allele leading to an excess of homozygotes). Excluding locus *MTT-8* from Hardy–Weinberg equilibrium tests in fact resulted in a decline of significant deviations to seven populations with the *P*-value for the Czech cultured strain from Hluboka (0.045) being only marginally significant (Table 3). After sequential Bonferroni corrections (initial *P*-value of 0.05 divided by nine tests = adjusted *P*-value of 0.0055) only three cultured strains still expressed significant deviations: German Königswartha, and Czech Marianske Lazne and Vodnany, both year classes 1996 and 1998. Subsequent tests of these strains for deficiency or excess of heterozygotes revealed highly significant ($P = 0.000$) excesses of heterozygotes in only two of them: Königswartha ($F_{IS} = -0.211$) and Vodnany, year class 1996 ($F_{IS} = -0.304$).

The evaluation of genetic differentiation between populations by F_{ST} values (Table 4) showed only ten non-significant differences between pairs of populations after sequential Bonferroni corrections (initial *P*-value of 0.05 divided by 210 possible tests = adjusted *P*-value of 0.00024). The Polish cultured strain from the fish farm in Szczodre was involved in five of these tests: it was not significantly differentiated

Table 3 Genetic variability parameters of 21 tench populations based on seven polymorphic microsatellite loci, results of Hardy–Weinberg probability tests based on nine or eight loci, and F_{IS} values based on eight loci

Population label	Average number of alleles	Number of private alleles	Observed heterozygosity (7 loci)	*P*-value of Hardy–Weinberg test	*P*-value of Hardy–Weinberg test[c]	F_{IS} values[c]
Doel[b]	4.29	2	0.488	0.046	0.082	0.015
Felc[a,b]	5.86	3	0.471	0.008	0.449	0.064
Kowa	3.57	1	0.611	0.000	0.000	−0.211
Ta98[b]	2.86	–	0.496	0.996	0.983	−0.076
ML98[a,b]	3.00	1	0.422	0.000	0.001	−0.056
Vo98[a,b]	2.57	–	0.351	0.000	0.002	−0.112
Vo96	2.43	1	0.526	0.000	0.000	−0.304
VoHy[a,b]	3.71	–	0.504	0.002	0.126	−0.133
VeMe[a,b]	4.86	1	0.443	0.000	0.618	0.057
Hlub[a]	5.29	2	0.454	0.000	0.045	0.078
Gold[a,b]	2.57	–	0.371	0.022	0.718	−0.126
Blue[b]	2.57	–	0.377	0.105	0.269	−0.065
Ital	5.14	3	0.471	0.012	0.012	−0.002
Bada[b]	1.00	–	0.000	–	–	–
Chin	1.71	4	0.074	1.000	1.000	−0.023
Roma[a,b]	3.43	–	0.422	0.000	0.486	−0.045
Hung[a,b]	2.71	–	0.352	0.000	0.019	−0.028
Turk[b]	1.29	1	0.034	0.864	0.864	0.052
Poli[a,b]	3.29	–	0.367	0.005	0.347	−0.041
Szcz[b]	4.00	–	0.464	0.831	0.680	0.038
NoWi[a,b]	3.29	1	0.459	0.013	0.070	0.009

[a] Populations with indication for presence of null allele(s) at locus *MTT-8*

[b] Populations being monomorphic at locus *MTT-3*

[c] Locus *MTT-8* excluded because of possible null allele(s)

from the German wild tench caught in lakes Döllnsee and Felchowsee, and from Italian cultured tench from Ceresole d′Alba, and Polish cultured strains from Poliwoda and Nowa Wieś. Non-significant differentiation was also observed between the Turkish wild tench and Spanish and Chinese cultured strains as well as between the two Czech cultured strains from Hluboka and Velke Mezirici, and between the golden and blue tench.

The generally significant population differentiation was also reflected by a relatively high accuracy of the assignment test: 69.3% or 549 out of the 792 tench individuals examined could be correctly classified according to the population of their origin (Table 5). At the population level the percentage of correctly classified individuals ranged from as low as 26.0% (Turkish wild tench) to as high as 100.0% (Spanish cultured strain). Remarkable misclassification was only observed among the less differentiated Czech cultured strains from Hluboka, Velke Mezirici and Vodnany hybrid, the Turkish wild and Chinese cultured tench from which large numbers were classified as Spanish cultured tench, but also in German wild tench from lake Felchowsee.

The D_A genetic distances displayed large variation among pairs of populations (Table 4). Surprisingly, the smallest distances were found between the geographically most distant populations: 0.008 between Turkish wild and Spanish cultured tench, 0.015 between Spanish and Chinese cultured tench, and 0.023 between Turkish wild and Chinese cultured tench. In contrast, genetic distances larger than 0.500 were measured between Italian cultured tench and Turkish wild tench ($D_A = 0.507$), Spanish cultured

Table 4 F_{ST} values (above diagonal; non-significant values in bold) and D_A distances (below diagonal) between pairs of 21 tench populations

	Doel	Felc	Kowa	Ta98	ML98	Vo98	Vo96	VoHy	VeMe	Hlub	Gold	Blue	Ital	Bada	Chin	Roma	Hung	Turk	Poli	Szcz	NoWi
Doel	–	0.024	0.091	0.093	0.073	0.169	0.147	0.074	0.088	0.065	0.171	0.187	0.075	0.764	0.637	0.144	0.297	0.724	0.081	**0.016**	0.044
Felc	0.061	–	0.083	0.064	0.080	0.141	0.120	0.059	0.086	0.060	0.138	0.140	0.048	0.616	0.526	0.114	0.253	0.589	0.066	**0.004**	0.035
Kowa	0.110	0.091	–	0.117	0.174	0.244	0.217	0.128	0.162	0.141	0.177	0.181	0.108	0.678	0.596	0.203	0.295	0.654	0.134	0.069	0.109
Ta98	0.110	0.106	0.120	–	0.126	0.112	0.126	0.036	0.092	0.083	0.134	0.178	0.124	0.648	0.545	0.063	0.209	0.619	0.101	0.056	0.096
ML98	0.112	0.112	0.171	0.126	–	0.137	0.136	0.090	0.108	0.092	0.155	0.212	0.123	0.647	0.548	0.119	0.279	0.623	0.152	0.093	0.092
Vo98	0.131	0.124	0.184	0.095	0.081	–	0.086	0.048	0.077	0.074	0.148	0.251	0.254	0.582	0.480	0.055	0.198	0.554	0.207	0.157	0.146
Vo96	0.174	0.146	0.206	0.174	0.143	0.106	–	0.070	0.108	0.096	0.145	0.200	0.208	0.623	0.528	0.096	0.250	0.599	0.175	0.140	0.108
VoHy	0.091	0.070	0.111	0.073	0.074	0.053	0.085	–	0.047	0.030	0.095	0.128	0.151	0.562	0.465	0.051	0.190	0.533	0.133	0.064	0.065
VeMe	0.085	0.076	0.118	0.114	0.111	0.080	0.139	0.045	–	**0.019**	0.100	0.177	0.172	0.419	0.328	0.043	0.112	0.390	0.136	0.099	0.087
Hlub	0.079	0.065	0.097	0.104	0.103	0.081	0.108	0.039	0.026	–	0.122	0.155	0.169	0.472	0.377	0.053	0.184	0.444	0.154	0.081	0.055
Gold	0.152	0.135	0.145	0.160	0.130	0.104	0.120	0.070	0.090	0.094	–	**0.169**	0.218	0.786	0.634	0.101	0.180	0.734	0.192	0.144	0.144
Blue	0.161	0.135	0.175	0.194	0.136	0.149	0.145	0.087	0.139	0.123	0.071	–	0.244	0.829	0.695	0.191	0.295	0.778	0.258	0.187	0.183
Ital	0.102	0.062	0.103	0.144	0.147	0.186	0.222	0.132	0.138	0.140	0.204	0.206	–	0.762	0.666	0.208	0.337	0.733	0.054	**0.034**	0.108
Bada	0.396	0.387	0.471	0.363	0.313	0.208	0.304	0.265	0.220	0.245	0.271	0.321	0.526	–	**0.079**	0.502	0.509	**0.096**	0.849	0.801	0.691
Chin	0.395	0.392	0.469	0.356	0.320	0.216	0.309	0.266	0.221	0.247	0.279	0.328	0.529	0.015	–	0.401	0.399	**0.075**	0.728	0.674	0.578
Roma	0.124	0.104	0.148	0.063	0.084	0.054	0.132	0.042	0.055	0.059	0.109	0.138	0.162	0.229	0.226	–	0.123	0.479	0.161	0.134	0.123
Hung	0.226	0.204	0.223	0.156	0.167	0.110	0.208	0.121	0.107	0.129	0.142	0.197	0.284	0.194	0.196	0.071	–	0.472	0.282	0.288	0.289
Turk	0.389	0.370	0.454	0.353	0.311	0.210	0.310	0.257	0.208	0.233	0.277	0.325	0.507	0.008	0.023	0.228	0.188	–	0.805	0.758	0.658
Poli	0.111	0.108	0.136	0.129	0.160	0.136	0.197	0.119	0.114	0.133	0.135	0.190	0.101	0.436	0.442	0.126	0.207	0.433	–	**0.039**	0.120
Szcz	0.059	0.032	0.072	0.096	0.128	0.115	0.167	0.082	0.083	0.079	0.121	0.154	0.068	0.404	0.409	0.119	0.227	0.390	0.074	–	**0.031**
NoWi	0.111	0.082	0.108	0.140	0.140	0.119	0.122	0.077	0.085	0.069	0.105	0.155	0.147	0.338	0.344	0.125	0.229	0.329	0.154	0.081	–

Table 5 Results of the assignment test by self-classification of 792 tench from 21 populations

Original population	Number of individuals classified into population …																				% Correct
	Doel	Felc	Kowa	Ta98	ML98	Vo98	Vo96	VoHy	VeMe	Hlub	Gold	Blue	Ital	Bada	Chin	Roma	Hung	Turk	Poli	Szcz	NoWi	
Doel	14				1					1			1							2		73.7
Felc	1	21	2					2		3	2	1	2						1	11	3	42.9
Kowa	2	1	44					1		1											1	88.0
Ta98				33		3		1								2	1					82.5
ML98					36	3			1	1						1						85.7
Vo98			1			42	1	2									4					84.0
Vo96						2	46	1			1											92.0
VoHy			1	3	1	4	1	22	3	5	2	1				4			1		2	44.0
VeMe	3	1		1		1		3	25	4	3		2	2		2			1		2	50.0
Hlub	2		5			5		1	5	24	1	1				2	2				2	48.0
Gold							1				14											93.3
Blue						1			1			13										86.7
Ital			1							1			27							1		90.0
Bada														50								100.0
Chin														18	13							41.9
Roma				3		6		4	1	3			1			30	1		1			60.0
Hung	1					2		2								1	35					85.4
Turk														37				13				26.0
Poli													2			1			11			78.6
Szcz		1	1			1							1						2	9	1	56.3
NoWi									1	2											27	90.0

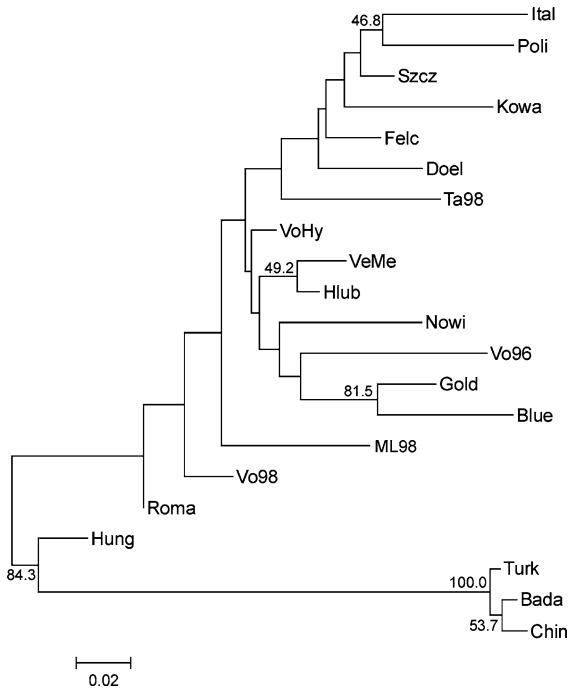

Fig. 1 Neighbor-Joining tree of 21 tench populations based on D_A genetic distances (Nei et al. 1983) and 1,000 bootstrap replicates (only bootstrap support higher than 40% is displayed on tree nodes)

tench ($D_A = 0.526$), and Chinese cultured tench ($D_A = 0.529$).

The Neighbor-Joining tree based on D_A genetic distances showed only two major clades supported by a high bootstrap value of 84.3% and consisting of four and 17 populations, respectively (Fig. 1). Within the smaller clade the Turkish wild tench and Spanish and Chinese cultured tench formed a sub-cluster with 100% bootstrap support. Another population pair with a high bootstrap support of 81.5% consisted of the golden and blue colour varieties.

Discussion

The two microsatellite loci *MTT-4* and *MTT-7* did not show any variability in all 792 tench individuals analysed so far. Therefore, it seems to be very unlikely that these loci are polymorphic at all. In order to save time, labour and costs they could be excluded from further studies.

Extending the number of tench populations/strains examined as well as the range of their geographic origin resulted in the detection of "new" alleles at some microsatellite loci compared to our previous study (Kohlmann et al. 2007): the total number of alleles across the nine loci increased from 49 to 66. Some of these new alleles were private ones leading to a rise in their number from 14 (previous study) to 20 (present study). On the other hand, at loci *MTT-1* and *MTT-9* the number of private alleles even declined from two to one and from seven to five, respectively, due to the fact that former private alleles were now recorded in more than one population/strain.

The general microsatellite variability of tench as found in the present study is comparable to that of other freshwater fish species. DeWoody and Avise (2000) conducted a literature search in May 1999 covering 524 microsatellite loci examined in nearly 40 000 individuals of 78 fish species and non-piscine animals, and reported mean heterozygosities of $h = 0.46 \pm 0.34$ at the population level and $H = 0.54 \pm 0.25$ at the species level, and mean numbers of alleles per locus of $a = 7.5 \pm 8.1$ at the population level and $A = 9.1 \pm 6.1$ at the species level, respectively. The corresponding values for marine fish species were considerably higher: $h = 0.79 \pm 0.26$, $H = 0.77 \pm 0.19$, $a = 20.6 \pm 11.8$ and $A = 19.9 \pm 6.6$. Anadromous fish species took an intermediate position: $h = 0.68 \pm 0.22$, $H = 0.68 \pm 0.12$, $a = 11.3 \pm 10.1$ and $A = 10.8 \pm 7.2$ (mean values $\pm$ SD).

After exclusion of the microsatellite locus *MTT-8*, which probably possesses null allele(s), and sequential Bonferroni corrections, all of the wild populations and all but three of the cultured tench strains were found to be in Hardy–Weinberg equilibrium. The highly significant overall excess of heterozygotes in the German strain from Königswartha and the Czech strain from Vodnany, year class 1996 might be caused by non-random "selection" of spawners used to produce the progeny from which muscle tissue samples were collected for analysis. Moreover, the breeding history of the tench from Königswartha is not known. Therefore, mixing of it with other strains and possible crossbreeding as a source of heterozygote excess cannot be excluded. On the other hand, the breeding history of the Vodnany tench is well documented. It has been bred in two lines, starting in 1979 (Kvasnička and Linhart 1990). The first line was based on two successive meiotic gynogenetic generations from which the $MeiG_2$ females were then mated to not-manipulated Vodnany males, which

gave origin of the Vodnany 1992 population. Its progeny was the Vodnany year class 1998 population examined in the present study. The second line has undergone normal mating but due to high mortalities, the remaining few individuals were mated to the first line in the 1990s. The Vodnany year class 1996 were progeny of Vodnany tench (3 females × 3 males) used for normal production, not for selection. Thus, hybridisation of the two lines might at least partly explain the observed excess of heterozygotes.

In contrast to our previous study (Kohlmann et al. 2007) including only two wild populations and four cultured strains from Central Europe no clear trends towards a reduction in genetic variability within and an increase in differentiation between cultured strains in comparison to wild populations could be observed in the present study. One wild tench population (Turkish Sapanca Lake) is among the least variable and is even less variable than most of the cultured strains while some cultured strains (Czech Hluboka and Italian Ceresole d′Alba) are similar to the most variable wild population (German lake Felchowsee). The two Czech cultured strains from Hluboka and Velke Mezirici show similar low levels of differentiation (F_{ST} value of 0.019) as the two German wild populations (F_{ST} value of 0.024), but are less differentiated from each other than the two German and Turkish wild tench (F_{ST} values of 0.724 and 0.589, respectively). These observations may reflect different breeding practices resulting in different effective population sizes in the cultured strains. The Italian strain for example is reproducing naturally with a probably higher number of randomly mating spawners than used in artificially reproduced strains. On the other hand, the tench has been domesticated for a shorter period of time with less farming intensity than the common carp in which a highly significant lower allelic richness at four microsatellite loci has been found in 13 domesticated/captive stocks (average $A_r = 4.436$) compared to nine wild-caught populations (average $A_r = 8.221$; Kohlmann et al. 2005). However, all present data on genetic variability and differentiation might be confounded by different degrees of mixing/secondary contact or even hybridisation of two divergent phylogenetic lineages of tench being present in Western and Central Europe. Nevertheless, for the cultured tench strains monitoring of their genetic variability is recommended to avoid losses of diversity in the future as already happened in other farmed fishes, e.g. the common carp. 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). In cultured strains with very low genetic variability higher levels can be recovered by controlled crossbreeding, i.e. accompanied by monitoring the performance (growth, survival, meat quality etc.) of the new synthetic strain. Uncontrolled crossbreeding might have detrimental effects such as loss or disruption of genetic adaptations to specific local environmental conditions and should therefore be avoided.

The unexpected grouping of tench from Spain, Turkey and China into one sub-cluster with 100% bootstrap support cannot be explained by the present microsatellite data alone. However, considering the results of the phylogeographic study by Lajbner et al. (2007) based on mitochondrial DNA and intron sequences of several nuclear genes they are in good agreement with the discovery of two highly divergent lineages of tench being present in Europe. The group formed by these three Spanish, Turkish and Chinese populations may represent the pure eastern lineage and is the sister group to the rest of the tree. The rest of populations might be mixtures between western and eastern lineages and the percentage of western alleles/genotypes probably determines most of the distance from the pure eastern group. Further studies combining nuclear (e.g. microsatellite) and mitochondrial DNA markers with morphological analysis and investigations on the origin of Spanish, Turkish and Chinese tench are needed to verify this conclusion.

A mixed nature is additionally supported for the wild tench population from the German lake Felchowsee by the relative low accuracy of the assignment test: only 21 out of 49 (=42.9%) of these tench were correctly classified into the population of their origin but 15 were assigned to Polish, eight to Czech, two to Italian and two to German cultured strains, and one individual to the second German wild population from lake Döllnsee. It has to be stressed, however, that no stocking with tench took place in lake Felchowsee (T. Löwe, personal communication).

The common grouping of the blue and golden tench varieties reflects their common ancestry, which

confirms the data of Kvasnička et al. (1998) who mated golden with blue tench in order to obtain wild-coloured, blue, golden and alampic progeny in F_2 and also backcrossed the wild-coloured F_1 to their ornamental parents in order to verify the Mendelian colour inheritance in the ornamental tench.

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