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Distribution of five growth hormone gene haplogroups in wild and cultured tench, *Tinca tinca* L., populations

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Summary

Tench, *Tinca tinca* L., has a growth hormone (GH) gene of 1758–1763 bp in length with at least 13 different haplotypes and 14 polymorphic sites (PS). Fragment length analysis at PS 1 and 7 with indels, and PCR-RFLP analysis at PS 5 and 13 with single nucleotide polymorphisms (SNPs) distinguish five GH gene haplogroups (H1-H3 belonging to the Western, W, and H4-H5, to the Eastern, E, phylogroup). Using quick and inexpensive screening assays, the aim of the study was to determine the allelic and genotypic distribution of the five GH gene haplogroups across 16 wild and cultured tench populations originating from all major parts of its distribution range. The mean observed number of haplogroups per population was 3.125. The most frequent haplogroup was H5 (0.482); the least frequent was H4 (0.022). The W haplogroups were observed in 12 out of the 16 populations, but no population had only the W haplogroup. With two exceptions (Ital and Kowa), the E haplogroups were observed in most populations as well as unique in three populations (Chin, Bada and Turk). Hardy–Weinberg equilibrium tests revealed heterozygote excess ($P < 0.05$) in four populations (Felc, ML98, Ta98 and Vo98) whereas heterozygote deficiency was not observed. Overall *F*-statistics showed quite a high degree of differentiation ($P < 0.01$) of tench populations, as the global F_{ST} was 0.212. The pairwise F_{ST} values ranged from 0.0 to 0.726. Non-significant differences between pairs of populations were observed in 41 cases (36.6%). Nei's standard genetic distances displayed large variations, ranging from 0.0 (between Bada and Chin) to 2.572 (between Turk and Ital). In some cases, similarities between populations from distant countries and dissimilarities between populations from the same country were observed. The Neighbour-Joining tree based on Nei's standard genetic distances showed two major clades corresponding with the observed frequencies of haplogroups within populations.

Introduction

The tench, *Tinca tinca* L., is a Eurasian freshwater fish species typically inhabiting shallow, densely vegetated lakes and backwaters (www.fishbase.org). Reared in ponds of Central and Eastern Europe as a so-called secondary or by-fish for hundreds of years (Steffens, 1995), its recent culture has expanded to China (Wang et al., 2006). However, due to slow growth, the tench has never attained a similar economic importance in aquaculture as the common carp, *Cyprinus carpio* L., with which it is often raised in bi-culture and/or polyculture. On the other hand, current attempts to diversify pond fish production have also led to an increased interest in tench. Considerable genetic differentiation among tench populations (Kohlmann et al., 2010; Lo Presti et al., 2010, 2012; Lajbner et al., 2011) as well as differences in the growth performance of tench strains (Gela et al., 2010; Kocour et al., 2010) has been documented. Thus, genetic improvement of the tench growth rate seems feasible.

Potential candidate genes of the somatotropic axis correlated with growth in livestock and fish include those coding for growth hormone-releasing hormone (GHRH), growth hormone inhibiting hormone (GHIH or somatostatin), growth hormone (GH), insulin-like growth factors (IGF-I and -II), and associated carrier proteins and receptors (De-Santis and Jerry, 2007). In a previous study (Kocour and Kohlmann, 2011) we focused on the detection and characterization of DNA sequence polymorphisms in the tench GH gene, since associations of GH genotypes with fish growth have already been investigated, e.g. in Atlantic salmon, *Salmo salar* (Gross and Nilsson, 1999), olive flounder, *Paralichthys olivaceus* (Kang et al., 2002) and Arctic charr, *Salvelinus alpinus* (Tao and Boulding, 2003). The complete tench GH gene was found to be 1758–1763 bp in length; altogether 13 different haplotypes with 14 polymorphic sites (PS) were observed, 12 located in introns (SNPs and very short indels) and two in exons (synonymous SNPs only). Based on these results we established a simple fragment analysis at PS 1 and 7 with indels, and a PCR-RFLP analysis at PS 5 and 13 with SNPs that may distinguish five haplogroups (named H1 to H5) of the GH gene. Three of the haplogroups (H1-H3) belong to the GH gene class I, and two (H4, H5) to the GH gene class II as defined by Kocour and Kohlmann (2011). These latter two GH gene classes correspond to the two previously detected tench phylogroups, Western and Eastern (Lajbner and Kotlik, 2011).

The aim of the present study was to determine the allelic and genotypic distribution of the five GH gene haplogroups across wild and cultured tench populations originating from all major parts of its distribution range using quick and

inexpensive screening assays and to assess the importance of the results for tench conservation and breeding.

Material and methods

For the present study, 16 of the 21 tench populations investigated by Kohlmann et al. (2010) for microsatellite-based genetic variability were taken. These tench originated from Spain, Italy, Germany, Czech Republic, Hungary, Romania, Turkey and China. Population details are given in Table 1. With the exception of the Blue population where only 15 samples were available, some 28–32 individuals were examined from each tench population.

Genomic DNA was isolated using the peqGOLD Tissue DNA MiniKit (Peqlab Biotechnologie). Primers for amplification of target tench GH gene fragments including the selected polymorphic sites (PS 1, 5, 7 and 13) were designed from a known GH gene sequence (GenBank accession no. GU205383) by PRIMER 3 PLUS software (Untergasser et al., 2007). The quality of primers was evaluated by NETPRIMER (http://www.premierbiosoft.com/netprimer/netprlaunch/netprlaunch.html). Forward primers for PS 1 and PS 7 were labelled with D3-PA and D4-PA dye (Sigma-Aldrich), respectively. The optimized PCR reaction mix composed 1.5 μl of 10 $\times$ 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^{-1}), 0.1 μl of *Taq* DNA-polymerase (5 units μl^{-1}; MBI-Fermentas), 2 μl of DNA template and sterile water to a final volume of 15 μl. The PCR protocol began with an initial denaturation step at 95°C for 3 min followed by 35 cycles consisting of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 45 s and a final extension step at 72°C for 7 min. PCR products were quality checked by electrophoresis on 1.7% agarose gels (0.5 $\times$ TBE buffer), stained with Ethidiumbromide and visualized on the Molecular Imager Gel Doc XR System (Bio-Rad Laboratories). Polymorphisms at PS 1 and 7 were analysed by fragment length determination on an automated capillary sequencer CEQ 8000 (Beckman Coulter); polymorphisms at PS 5 and 13 were analysed by electrophoretic separation of restriction fragments on 2.5% agarose gel (0.5 $\times$ TBE buffer) stained with Ethidiumbromide after enzyme digestion at 37 or 60°C using reaction mixtures according to the recommendations of the restriction enzyme supplier (MBI-Fermentas). Enzymes used for detection of polymorphisms were Tru1I for PS 5 and Hph1 and TscAI for PS 13 (Table 2). Haplogroups were determined as shown in Table 3.

After assigning the GH gene of each individual to haplogroups, the population genetic software MSA (Dieringer and Schlötterer, 2003) was used to calculate the (i) haplogroup (allelic) frequencies and phylogroup (genotypic) frequencies (W – Western only, H – hybrid, E – Eastern only) for each population, (ii) F_{ST} values, with testing their significance by bootstrap analysis (1000 replicates), and (iii) Nei's standard genetic distances between populations (Nei, 1987). The matrix of genetic distances was then used in order to construct a Neighbour-Joining tree with the MEGA 4 program (Tamura et al., 2007). Hierarchical partition of genetic diversity was evaluated by analysis of molecular variance (AMOVA) using ARLEQUIN v.3.5 (Excoffier et al., 2005).

Results

All individuals were successfully assigned to the haplogroups screened. The number of alleles, representing different GH gene haplogroups, observed within the tench populations varied from one (Bada and Chin) to four (Blue, Doel, Felc, Hlub, Ital, Kowa, Roma, and VeMe). Thus, the mean number of alleles per population was 3.125. The most frequent across all populations were H5 (0.482) and H1 (0.258); less frequent were H3 (0.086) and H4 (0.022). Allele H5 was found within all tench populations, but allele H4 only in the wild Turkish population (Table 3).

Table 1
Description of 16 tench populations examined for variation of five GH gene haplogroups

Population label	Full name	n	Brief characterization
Doel	Döllnsee	28	German wild population from a lake
Felc	Felchowsee	31	German wild population from a lake
Kowa	Königswartha	32	German cultured strain[a]
Ta98	Tabor	31	Czech cultured breed[b], year class 1998
ML98	Marianske Lazne	30	Czech cultured breed[b], year class 1998
Vo98	Vodnany	32	Czech cultured breed[b], year class 1998
Vo96	Vodnany	32	Czech cultured breed[b], year class 1996
VeMe	Velke Mezirici	30	Czech cultured breed[b]
Hlub	Hluboka	31	Czech cultured breed[b]
Blue	Blue	15	Colour variety developed in Vodnany
Ital	Italy	30	Ceresole d'Alba, cultured strain[a], natural reproduction
Bada	Badajoz	31	Spanish cultured strain[a] collected at Las Vegas del Guadiana fish farm
Chin	China	30	Chinese cultured strain[a] collected in Wuhan originating from a few broodfish in northwest China
Roma	Romania	31	Romanian cultured strain[a] collected at Vodnany live gene bank
Hung	Hungary	31	Hungarian cultured strain[a] collected at Vodnany live gene bank
Turk	Turkey	31	Turkish wild population caught in Sapanca Lake

n, number of analysed individuals.
[a]Term 'strain' = cultured stocks not registered as a breed and/or history of origin unknown.
[b]Term 'breed' = cultured stocks officially registered on list of breeds of the given country and/or have clear history of origin.

Table 2
Description of polymorphic sites (PS) in tench GH gene used to identify five haplogroups

PS	PT	PCR primers to amplify the fragment 5′–3′	Method of screening	Observed length of fragments (bps)	Scoring
1	Indel	F – GGATGTGATGCTTTCTGTGG	Fragment analysis	341 or	Absent
		R – AAGAGCCGCTGGTTCTCTG		344 or	Present
				341 and 344	Both
5	SNP	F – CGACTTTGTAAGATTTCCATTCA	PCR-RFLP (enzyme Tru1I)	97, 55 or	C/C
		R – TCGAGGGTGAGCAAATGATA		80, 55 or	T/T
				97, 80, 55	C/T
7	Indel	F – CGACTTTGTAAGATTTCCATTCAA	Fragment analysis	451 or	Absent
		R – GCAACAGGTTGTCCTCCTTG		455 or	Present
				451 and 455	Both
13	SNP	F – TGCT YAGAAATCAGAAAATGCT	PCR-RFLP (enzymes	311 (Hph1) and 311 (TscAI)	A/A
		R – AAATCTGGTCTCTCTCACCTTGA	Hph1 and TscAI)	248, 63 (Hph1) and 311 (TscAI)	C/C
				311 (Hph1) and 248, 63 (TscAI)	T/T
				311, 248, 63 (Hph1) and 311 (TscAI)	A/C
				311 (Hph1) and 311, 248, 63 (TscAI)	A/T
				311, 248, 63 for both (Hph1 and TscAI)	C/T

PT, polymorphism type; Indel, insertion/deletion; SNP, Single nucleotide polymorphism; F, forward primer; R, reverse primer; A, C, T, individual nucleotides.

Table 3
Identification of haplogroups based on results of fragment analysis at PS 1 and 7 and PCR-RFLP analysis at PS 5 and 13

			Fragment length				
Haplogroup (H)	Phylogroup	Haplotypes included*	PS 1	PS 5	PS 7	PS13 – Hph1	PS13 – TscAI
1	W	1, 4	344	97 and 55	451	248 and 63	311
2	W	2, 3, 5, 6	344	97 and 55	451	311	248 and 63
3	W	7, 8	344	80 and 55	451	311	248 and 63
4	E	9	344	97 and 55	455	311	311
5	E	10 – 13	341	97 and 55	455	311	311

W, Western phylogroup; E, Eastern phylogroup; Hph1 and TscAI, restriction enzymes.
*For details of GH gene sequences see Kocour and Kohlmann (2011).

From the 15 theoretically possible GH gene haplogroup genotypes, 12 were recorded. The most common genotype H5H5 with a frequency of 0.279 appeared in all populations but two (Ital and Kowa); on the other hand, the rarest genotypes, H3H3 with a frequency of 0.002 and H4H4 with a frequency of 0.008, appeared in only one population (Hlub and Turk, respectively).

Western genotypes (with alleles H1-H3 at both chromosomes) were observed in 12 out of the 16 populations but no population exhibited only the Western genotype). Eastern genotypes (with alleles H4-H5 at both chromosomes) were also observed in most populations except for Ital and Kowa. Unlike the Western, the Eastern genotypes were unique for three populations (Chin, Bada and Turk). The heterozygous genotype with respect to phylogroup origin (genotypes with alleles H1-H3 at one chromosome and allele H4-H5 at the second chromosome) was found in all populations except those with uniquely fixed Eastern genotypes (Table 4).

Overall *F*-statistics of the tench GH gene polymorphisms showed that populations are quite highly differentiated ($P < 0.01$), as global F_{ST} was 0.212. On the other hand, global F_{IS} and F_{IT} values (−0.083 and 0.147, respectively) showed that inbreeding within tench populations does not exist and that overall inbreeding (deviation from non-random mating for the species) is caused by the separation (isolation) of tench populations. Results of overall *F*-statistics corresponded to the Hardy–Weinberg equilibrium tests, which revealed heterozygote excess ($P < 0.05$) in four populations (Felc, ML98, Ta98 and Vo98) whereas heterozygote deficiency was not observed in any population. Only the population of Blue tench was very close to the threshold of significance for heterozygote deficiency ($P = 0.0572$). The pairwise evaluation of genetic differentiation between tench populations revealed F_{ST} values from 0.0 (no difference) between Bada and Chin up to 0.726 between the Blue and Bada populations (Table 5). Non-significant differences between pairs of populations after sequential Bonferroni corrections were observed in 41 cases (36.6% of all pairs).

The pairwise Nei's standard genetic distances (Table 5) displayed large variation and ranged from 0.0 (between Bada and Chin) to 2.572 (between Turk and Ital). Similarity between Bada and Chin populations was quite surprising, also the very low genetic distances between some tench populations seemingly to originate from different countries (e.g. Roma vs Doel and Blue vs Kowa with $D = 0.011$ and Hung vs ML98 and Roma vs Hlub with $D = 0.014$). Conversely, some populations with origin in the same country (e.g. Blue vs Vo96, Blue vs Vo98, ML98 vs Ta98) and the same

Table 4
Allelic and genotypic frequencies of GH gene haplogroups observed in 16 tench populations

	Allelic frequencies					Genotypic frequencies											
	W			E		W						H			E		
Pop.	H1	H2	H3	H4	H5	H1 H1	H1 H2	H2 H2	H1 H3	H2 H3	H3 H3	H1 H5	H2 H5	H3 H5	H4 H4	H4 H5	H5 H5
Blue	0.567	0.233	0.033	–	0.167	0.333	0.200	0.133	0.067	–	–	–	0.200	–	–	–	0.067
Chin	–	–	–	–	1.000	–	–	–	–	–	–	–	–	–	–	–	1.000
Doel	0.536	0.071	0.018	–	0.375	0.321	0.107	–	–	–	–	–	0.321	0.036	0.036	–	0.179
Felc	0.419	0.177	0.210	–	0.194	0.032	0.226	0.032	0.355	–	–	–	0.194	0.065	0.065	–	0.032
Hlub	0.258	0.161	0.161	–	0.419	0.065	0.194	–	0.065	0.032	0.032	–	0.129	0.097	0.161	–	0.226
Hung	0.371	–	–	–	0.629	0.161	–	–	–	–	–	–	0.419	–	–	–	0.419
Ital	0.433	0.383	0.133	–	0.050	0.200	0.233	0.200	0.133	0.133	–	–	0.100	–	–	–	–
Kowa	0.453	0.328	0.016	–	0.203	0.188	0.313	0.063	0.031	–	–	–	0.188	0.219	–	–	–
ML98	0.233	-	0.100	–	0.667	–	–	–	–	–	–	–	0.467	–	0.200	–	0.333
Roma	0.387	0.129	0.032	–	0.452	0.194	0.097	0.032	-	0.032	–	–	0.290	0.065	0.032	–	0.258
Bada	–	–	–	–	1.000	–	–	–	–	–	–	–	–	–	–	–	1.000
Ta98	0.210	0.452	-	-	0.339	-	0.161	0.194	–	–	–	–	0.258	0.355	–	–	0.032
Turk	–	–	–	0.339	0.661	–	–	–	–	–	–	0.129	–	–	–	0.419	0.452
VeMe	0.300	0.267	0.150	–	0.283	0.100	0.233	-	0.067	0.133	–	–	0.100	0.167	0.100	–	0.100
Vo96	-	0.250	0.234	–	0.516	–	–	–	–	–	–	–	–	0.500	0.469	–	0.031
Vo98	0.156	0.250	–	–	0.594	0.031	–	–	0.031	–	–	–	0.219	–	0.469	–	0.250

W, Western phylogroup; E, Eastern phylogroup; H, hybrid phylogroup; H1-H3, Western alleles; H4-H5, Eastern alleles; H1H1-H3H3, Western genotypes; H1H5-H3-H5, Hybrid genotypes; H4H4-H5H5, Eastern genotypes.

Table 5
F_{ST} values (above diagonal) and Nei's standard genetic distances (below diagonal) inferred from distributions of five GH gene haplogroups

Pop.	Blue	Chin	Doel	Felc	Hlub	Hung	Ital	Kowa	ML98	Roma	Bada	Ta98	Turk	VeMe	Vo96	Vo98
Blue	–	0.722	0.029*	0.019*	0.094*	0.210*	0.021*	0.006*	0.270	0.064*	0.726	0.122*	0.410	0.043*	0.272	0.268
Chin	1.313	–	0.546	0.550	0.378	0.355	0.654	0.582	0.255	0.411	n.d.	0.508	0.323	0.478	0.352	0.295
Doel	0.050	0.550	–	0.054*	0.060*	0.068*	0.141	0.062*	0.138	0.006*	0.551	0.159	0.314	0.067*	0.236	0.169
Felc	0.025	1.001	0.087	–	0.039*	0.173	0.034*	0.031*	0.191	0.056*	0.554	0.109	0.326	0.009*	0.169	0.162
Hlub	0.240	0.238	0.110	0.106	–	0.069*	0.123	0.076*	0.057*	0.008*	0.382	0.067*	0.188	0.005*	0.052*	0.042*
Hung	0.355	0.142	0.081	0.319	0.070	–	0.300	0.198	0.016*	0.025*	0.359	0.210	0.203	0.140	0.189	0.083*
Ital	0.044	2.462	0.307	0.072	0.367	0.794	–	0.015*	0.323	0.142	0.658	0.094	0.425	0.043*	0.246	0.297
Kowa	0.011	1.061	0.107	0.060	0.192	0.363	0.029	–	0.239	0.062*	0.586	0.055*	0.358	0.022*	0.212	0.241
ML98	0.575	0.060	0.200	0.407	0.056	0.014	1.033	0.537	–	0.060*	0.259	0.212	0.148	0.143	0.117	0.020*
Roma	0.134	0.286	0.011	0.124	0.014	0.019	0.368	0.129	0.075	–	0.416	0.091*	0.218	0.031*	0.133	0.090
Bada	1.313	0.000	0.550	1.001	0.238	0.142	2.462	1.061	0.060	0.286	–	0.512	0.327	0.483	0.355	0.299
Ta98	0.270	0.561	0.351	0.291	0.160	0.392	0.215	0.110	0.424	0.199	0.561	–	0.290	0.033*	0.111	0.209
Turk	1.423	0.110	0.660	1.110	0.348	0.252	2.572	1.171	0.169	0.396	0.110	0.671	–	0.260	0.183	0.158
VeMe	0.088	0.571	0.122	0.017	0.012	0.228	0.099	0.038	0.259	0.062	0.571	0.063	0.681	–	0.091	0.121
Vo96	0.912	0.170	0.612	0.509	0.099	0.312	0.852	0.633	0.164	0.298	0.170	0.232	0.280	0.212	–	0.065
Vo98	0.731	0.100	0.317	0.394	0.057	0.098	1.080	0.679	0.015	0.158	0.100	0.510	0.210	0.264	0.095	–

n.d., no difference.
*F_{ST} value not significantly different from 0 (P = 0.05).

population from different year classes (Vo96 vs Vo98) showed quite high genetic distances (D = 0.912, 0.731, 0.424 and 0.095, respectively). The Neighbour-Joining tree based on Nei's standard genetic distances showed two major clades consisting of five and eleven populations (Fig. 1). The clades corresponded with observed frequencies of alleles within each population according to their phylogroup origin.

Discussion

Zhu et al. (1992) characterised the GH gene in grass carp, *Ctenopharyngodon idella*, and concluded that polymorphisms in this gene can be observed only in evolutionary tetraploid fishes. Indeed, first observations on GH gene polymorphisms were done on salmonids, which are evolutionary tetraploid and have six-exon GH genes (Forbes et al., 1994; Park et al., 1995; McKay et al., 1998; Spruell et al., 1999). Moreover, in fish species with six-exon GH genes, polymorphisms in mini- and/or microsatellite repeats in introns were often found (Ber and Daniel, 1993; Tanaka et al., 1995; Yowe and Epping, 1996; Almuly et al., 2000; Zhang et al., 2009). Later on, polymorphisms were also detected in GH genes of diploid fishes with five exons. Gross et al. (1996) identified a two-allele polymorphism within a 262 bp GH gene restriction

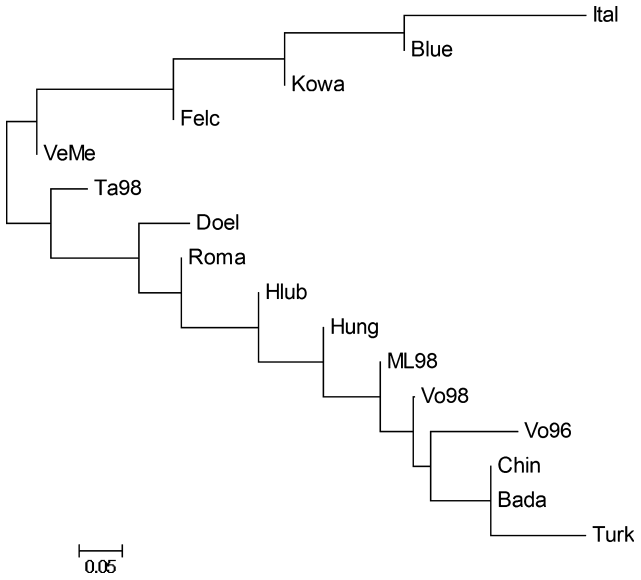

Fig. 1. Neighbour-Joining tree of investigated tench populations based on Nei's standard genetic distances (Nei, 1987)

fragment of common bream, *Abramis brama*, from the Main and Danube rivers and Kocour and Kohlmann (2011) found 14 polymorphic sites in the tench GH gene with 13 different haplotypes. It is evident that even diploid fish can show significant polymorphisms in any gene; the presence of polymorphisms depends on the phylogenetic history of the species and/or screening of sufficient numbers of fish and geographically distinct populations.

Most previous works did not include any true population genetic study of the species based on the GH gene polymorphisms in fish. Gross and Nilsson (1995) focused on the GH2 gene in brown trout (*Salmo trutta* L.) and, using heteroduplex analysis, identified two polymorphisms that were observed in four out of the six populations studied. Ryynänen and Primmer (2004) probably performed the only comprehensive study, whereby they used 17 polymorphic sites of the GH1 gene in nine populations of Atlantic salmon from Europe and North America. Results presented in our study concerned 16 wild as well as cultured tench populations and four polymorphic sites, which enabled us to characterize altogether five haplogroups with one to four haplogroups per population. The rarest tench GH haplogroup frequency was 0.022. In the study by Ryynänen and Primmer (2004) altogether 43 different haplotypes were observed with four to fourteen haplotypes per population, which is much more than in our study. However, when considering the haplotypes with a frequency higher than 0.022 the number of Atlantic salmon GH1 haplotypes was reduced to 11.

Several studies have recently focused on tench in order to study its population genetic structure (Kohlmann et al., 2010; Lajbner et al., 2011; Lo Presti et al., 2012). Many of the investigated populations were common to all studies, including ours. All studies describe the existence of two quite distinct subgroups. The subdivision is the existence of two distinct tench phylogroups – Eastern and Western (Lajbner and Kotlik, 2011) in which different haplotypes (alleles) for different genetic markers (actin, ATPase, S7 ribosomal protein and cytochrome b – Lajbner et al., 2011; ND-1, ND-6, D-loop and cytochrome b – Lo Presti et al., 2012; and the GH gene – Kocour and Kohlmann, 2011) were observed. Shown is that European tench populations – wild as well as cultured – are composed of individuals of both phylogroups and/or heterozygotes, whereas Asian populations have alleles of the Eastern phylogroup only. The Bada population from Spain was the exception among the European populations when having individuals with alleles of the Eastern phylogroup (haplogroup H5) only. The same population was 100% homozygous based on microsatellite analysis (n = 50) (Kohlmann et al., 2010) as well as PCR-RFLP analysis of mtDNA (n = 10) (Lo Presti et al., 2012). Thus, the Bada population is inbred and was probably established from a very low number of parents. Due to the presence of Eastern phylogroup alleles, the Bada population clustered together with Asian populations (Chin and Turk). On the other hand, the Turkish population had two different haplogroups in the present study (observed heterozygosity, $H_O = 0.419$), with similar results found by Lo Presti et al. (2012) based on PCR-RFLP analysis of mtDNA. However, based on the microsatellite analysis (Kohlmann et al., 2010) the Turkish population has very low H_O (0.034) because of a low average number of alleles per locus (1.29). Conversely, the Chinese population had $H_O = 0.074$ based on microsatellite analysis with an average number of alleles per locus 1.71, however, in our study and in the PCR-RFLP analysis of mtDNA by Lo Presti et al. (2012), the Chinese population was monomorphic. An overall comparison of the different genetic markers indicates that the tench GH gene displays a higher within-population variability than the PCR-RFLPs of mtDNA but lower variability than microsatellites.

Neighbour-Joining trees based on microsatellites (Kohlmann et al., 2010), PCR-RFLP analysis of mtDNA (Lo Presti et al., 2012) and the GH gene (Fig. 1) show similarities in the subdivision into two or three clusters based on the frequencies of alleles of the different phylogroups within each tench population. Because of the differences in frequencies for each genetic marker and also not fully identical sets of populations included in each study, the only and very evident character may be seen in the clustering of populations containing alleles of the Eastern phylogroup (Chin, Bada and Turk). The other tench populations clustered differently for different genetic markers. The best results for the tench population structure would be obtained when constructing the tree on the basis of all genetic markers combined.

As discussed above, tench exist in two divergent phylogroups. According to the sequence divergence between these phylogroups at mtDNA (1.3% for the cytochrome b gene), the two phylogroups may be phylogenetically considered as being different fish species (Lajbner et al., 2010). However, Lajbner et al. (2010) found no evidence for reproductive isolation between these two phylogroups in natural conditions. Tench populations with different phylogroup profiles (Eastern, Western, and mixed/hybrid) may display different overall phenotypes (morphology, physiology, behaviour, reproduction, growth, resistance, etc.). However as of the

present, there is a lack of data to assess the relationship in tench between the phenotype and the phylogroup. There is also no evident association between the growth performance and phylogroup. The test of growth performance under pond conditions by Kocour et al. (2010) showed a significantly higher corrected weight at market size in the Hluboka breed (302.1 ± 5.3^{c}; mean ± SE) compared to the Velke Mezirici (271.9 ± 3.5^{b}), Tabor (278.6 ± 3.6^{b}) and Vodnany (215.4 ± 5.2^{a}) breeds. The test of growth performance by Gela et al. (2010) showed significantly higher corrected weights at market size in the Königswartha breed (371.4 ± 174.0^{b}; mean ± SD) compared to the Hungarian (254.6 ± 66.1^{a}) and Vodnany (205.6 ± 65.8^{a}) breeds. When looking at the GH gene Neighbour-Joining tree of tench populations (Fig. 1), the GH gene haplogroup allele and genotype frequencies (Table 4), and results from performance tests, no speculation can be established. However, the precise GH genetic profile of tested populations was not known even when some conformity might be expected, because the tested populations but Königswartha came from parental stocks that were sampled for the present study. Several studies showed discordance between genetic structure and morphological, ecological, and/or physiological adaptation (Wilson et al., 2004; see also review by Okumus and Ciftci, 2003). A significant association between polymorphisms in the GH gene and growth has been observed in Atlantic salmon (Gross and Nilsson, 1999), olive flounder (Kang et al., 2002) and Nile tilapia (Blanck et al., 2009). In tench, another challenge is to run properly designed experiments in order to estimate the extent of association between the GH gene phylogroups/haplogroups and growth or other traits and to evaluate the practical implications of the results in breeding and conservation. The results of the present study might be useful for selecting populations with desired haplogroups and frequencies for further research. In each case, the distribution of phylogroups within tench populations used in any experiment should be taken into account.

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