Genetic characterization of introduced Tunisian and French populations of pike-perch (*Sander lucioperca*) by species-specific microsatellites and mitochondrial haplotypes

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ABSTRACT: The pike-perch is among the economically most valuable fish species for both commercial and recreational fishermen. This could be seen as the main reason for its introductions into Western Europe (including France) and Tunisia. Knowledge of the genetic structure of the introduced populations is a prerequisite for their successful long-term management. The present study focuses on the genetic characterization of introduced Tunisian and French pike-perch populations using species-specific microsatellite loci and mitochondrial cytochrome *b* haplotypes in order to better understand their genetic relationships and to try to trace the origin of the Tunisian populations. Lowered levels of genetic diversity have been observed in the two introduced Tunisian populations and a farmed Czech strain compared to a native wild German population. The reduction of microsatellite genetic variability of these three populations was supported by a genetic bottleneck signature. In contrast, the French populations showed high genetic diversity, probably due to multiple introductions and admixture of genetically differing sources. A high genetic differentiation level (significant F_{ST} values) between most pike-perch populations and a high average accuracy of self-assignments of individuals to populations of their origin were observed, probably resulting from genetic drift. The average pairwise relatedness values and results of the structure analysis highlighted a closer relationship between Tunisian and French populations than between Tunisian and German ones. Indeed, the two Tunisian populations clustered together with the French populations on a Neighbour-Joining tree based on D_A genetic distances. This was also sustained by the distribution of cytochrome *b* haplotypes A and B in the studied populations. The present results demonstrate that, despite the genetic differences, the studied populations cluster according to their phylogeographic origin. The Tunisian populations seem to be introduced from a French hatchery where the brood stock had the haplotype B of the mitochondrial cytochrome *b* gene.

Keywords: zander; nuclear markers; cytochrome *b*; stocking; North Africa; Europe

INTRODUCTION

The pike-perch (*Sander lucioperca*) is an Eurasian percid fish species that is widely distributed in watercourses. The limits of its native range extend from the Elbe River to Lake Onega in the north and from the Maritsa River to the Aral Sea in the south (Khurshut and Kohlmann 2009; Saisa

et al. 2010). Lappalainen et al. (2003) reviewed the current knowledge on the reproduction biology of pike-perch which also might influence the genetic structure of wild populations. Mature pike-perch conduct short spawning migrations (usually less than 35 km) both in fresh and brackish waters. A homing behaviour is well developed based on tagging experiments. Thus, even geographically close populations may be relatively isolated and may display significant genetic differentiation. Pike-perch are spawning in pairs and thus can be considered a monogamous species. Males are guarding and taking care of eggs what prevents them from fertilizing eggs of other females. Therefore, the effective population size and the resulting genetic variability within populations might be lower in comparison to mass-spawning fish species. Bjorklund et al. (2007) estimated an effective population size of only ca. 100 pike-perch individuals in two Fennoscandian regions (North and South). Moreover, allelic richness and the degree of differentiation based on microsatellite loci was significantly higher in the North (eight populations) than in the South (ten populations) suggesting that there has been more gene flow between pike-perch populations in southern areas than in northern areas, where the importance of genetic drift has been greater. Similarly, Saisa et al. (2010) found marked genetic differentiation between three coastal (=indigenous) and five lake (=introduced or mixed) populations of pike-perch from Finland. In general, the lake populations showed a higher genetic diversity than the coastal ones. Most recently, Eschbach et al. (2014) identified three genetic lineages of pike-perch in Germany corresponding to the river basins of the Danube, Oder, and Elbe. They also observed an increased genetic diversity and admixture in invaded regions (either due to stocking or migration through artificial canals) highlighting an asymmetric gene flow towards these areas. Thus, genetic drift and gene flow seem to be the most important factors determining the genetic variability and structure of wild pike-perch populations (either native or introduced).

The pike-perch is one of the main commercial species in countries where it is naturally occurring. In order to increase catches and establish new fishable populations, numerous introductions of pike-perch took place in many countries thereby expanding its geographical range. For example, it has been introduced in Western Europe into France, Belgium, the Netherlands and Great Britain, but also into Turkey, Morocco, and into some dams in the Maghreb countries and in the Azores islands (source: www.fishbase.org).

Despite of possible economic benefits for fisheries, the introduction of non-native species also has many disadvantages. Such human activities can damage the native biodiversity by the pressure exerted from the introduced species. Genetically, the stocking programs were generally based on a low number of pike-perch founder individuals, involved a population bottleneck, and resulted in a large-scale loss of the original genetic diversity within the species. As a result, the newly established populations are expected to display a low level of genetic diversity and a high risk of extinction (Allendorf and Lundquist 2003).

In Tunisia, the introduction of pike-perch began on the Nebhana dam in 1968 by the Office National des Pêches (ONP; National Fishery Office) when 30 000 eggs in incubating vats were imported. Then, in 1990, 24 breeders sampled from the Nebhana dam were stocked into the Sidi Salem dam reservoir. Since its introduction into the reservoir of Sidi Salem, pike-perch experienced a remarkable development. It represents, with 35% of the captured biomass, the most appreciated freshwater fish after mullets *Mugil cephalus* and *Liza ramada*.

In France, the pike-perch started invading at the end of the 19th century (Poulet et al. 2009). Since the middle of the 20th century, pike-perch populations increased all over the country including the strongly anthropic environments. Now pike-perch represents an important fisheries resource (Poulet et al. 2009).

In most stocking programs, no information is available for characterization of the batch that was initially introduced. However, detailed knowledge of the genetic diversity of the exploited fish populations is a prerequisite for their successful long-term management. Thus, the present study was aimed to characterize the genetic diversity of introduced pike-perch populations from Tunisia and France using microsatellite loci in order to better understand and estimate their genetic relationships, to trace the origin of the Tunisian populations, and to discuss the results in the light of the level of genetic diversity observed in native populations and published data available for pike-perch. For comparative reasons, populations from

some other European countries as well as published information on the distribution of mitochondrial cytochrome *b* haplotypes were also included.

MATERIAL AND METHODS

Sampling. Pike-perch fin clips were collected from 17 populations (2 introduced Tunisian populations, 12 introduced French populations, 1 German native wild population, and 2 farmed populations from the Netherlands and the Czech Republic) (Table 1). Genomic DNA was isolated from the fin clips using the peqGOLD Tissue DNA Mini Kit (Peqlab Biotechnologie GmbH, Erlangen, Germany).

Microsatellite genotyping. The nine pike-perch specific microsatellite loci *MSL-1* to *MSL-9* described by Kohlmann and Kersten (2008) were PCR-amplified on a Mastercycler gradient apparatus (Eppendorf AG, Hamburg, Germany). Primer sequences taken from the original publication can be found under GenBank accession numbers EF694018–EF694026. Each reaction mix was composed of 1.5 μl of 10× PCR buffer with $(NH_4)_2SO_4$ (MBI-Fermentas, Amherst, USA), 1.2 μl of 25mM $MgCl_2$, 1.2 μl of 1.25mM dNTPs, 0.3 μl of each primer (10 pmol/μl),

50–100 ng genomic DNA, 0.1 μl of *Taq* DNA-polymerase (5 units/μl; MBI-Fermentas), and sterile water up to a final volume of 15.0 μl. For cycling, a "touch down" thermal profile was used: initial denaturation at 94°C for 3 min, 10 cycles of 40 s at 94°C, 40 s at 60–50°C (1°C decrease per cycle), 1 min at 72°C, and 25 cycles of 40 s at 94°C, 40 s at 50°C, 1 min at 72°C, and a final extension for 10 min at 72°C. All nine loci were amplified separately, but to allow multiplex fragment analyses on a CEQ 8000 Genetic Analysis System (Beckman Coulter Inc., Fullerton, USA), the forward primer of each pair was labelled with one of three WellRed fluorescence dyes (Proligo®; Sigma-Aldrich, St. Louis, USA).

Microsatellite genotypes were recorded using the CEQ 8000 Genetic Analysis System, Fragment Analysis module (Beckman Coulter Inc.), and examined with MICRO-CHECKER v.2.2.3 (Van Oosterhout et al. 2004) for possible genotyping errors due to non-amplified alleles (null alleles), short allele dominance (large allele dropout), and the scoring of stutter peaks. This software estimates the frequency of null alleles and, importantly, can adjust the allele and genotype frequencies of the amplified alleles, permitting their use in further population genetic analysis.

Table 1. Sampled water bodies, their geographical coordinates, and sample sizes (*n*) of pike-perch

Water body	ID	Geographical coordinates	Pike-perch population	*n*
Sidi Salem (TUN)	SSL	36°39´N; 9°24´E	introduced: adults translocation (from Nebhana) in 1990	50
Nebhana (TUN)	NEB	36°03´N; 9°51´E	introduced: eggs seeding in 1968	54
Venlo (NTL)	NTL	–	farmed	41
Lake Müggelsee (DEU)	MUG	52°26'N; 13°38' E	wild, native	50
Vodňany (CZR)	CZR	–	farmed	23
Complexe d'Eguzon (FRA)	CEG	46°27'N, 1°36'E	introduced	31
Ardres (FRA)	ARD	50°53'N, 1°58'E	introduced	30
Etang du Puits (FRA)	EPU	47°35'N, 2°22'E	introduced	27
Lac de Pont (FRA)	LPO	47°28'N, 4°21'E	introduced	22
Lac de Crescent (FRA)	LCR	47°23'N, 3°54'E	introduced	37
Lac de Madine (FRA)	LMD	48°55'N, 5°44'E	introduced	30
Etang des Aulnes (FRA)	ETA	43°35'N, 4°47'E	introduced	30
Retenue de Saint-Ferréol (FRA)	RSF	43°26'N, 2°1'E	introduced	27
Retenue de Bage (FRA)	RBA	44°15'N, 2°43'E	introduced	30
Retenue du Tordre (FRA)	RTO	44°0'N, 1°27'E	introduced	30
Lac d'Aydat (FRA)	LAY	45°39'N, 2°59'E	introduced	16
Retenue de Hautefage (FRA)	RHA	45°4' N, 2°1'E	introduced	19

TUN = Tunisia, NTL = the Netherlands, DEU = Germany, CZR = Czech Republic, FRA = France
ID = sample location identification given by a three-letter code

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Genetic data analysis

Variability of pike-perch microsatellite loci and populations. The GENETIX 4.05 software (Belkhir et al. 1996) was used to calculate mean number of alleles per locus for each and all populations (A_m), observed (H_o) and unbiased expected (H_e) heterozygosity for each locus and mean heterozygosity. Allelic richness (A_r) and richness in private alleles (A_p) following a rarefaction method were calculated for each population using two programs: ADZE (Szpiech et al. 2008) and HP-Rare (Kalinowski 2005). Statistical analyses (one-way ANOVA) using the SPSS program (Version 10, 2001) were performed to test the differences between populations in mean values of A_r and A_p, with P values < 0.05 taken as indicating statistical significance.

Recent effective population size reductions (genetic bottlenecks) were studied using allele frequency data and BOTTLENECK software (Version 1.2.02) (Cornuet and Luikart 1996). To determine whether a population exhibits a significant number of loci with heterozygosity excess, BOTTLENECK proposes three tests: Sign test, Standardized differences test (minimum 20 loci), and Wilcoxon sign-rank test. Finally, the allele frequency distribution was established in order to see whether it is approximately L-shaped (as expected under mutation-drift equilibrium) or not (recent bottlenecks provoke a mode shift). As recommended by Piry et al. (1999), the Two Phase Mutation Model (TPM) with 95% proportion of the Stepwise Mutation Model (SMM) and 5% of the Multistep mutations was used.

Wright's inbreeding coefficient F_{IS} was computed for each population and for each locus, providing information on departure from the Hardy-Weinberg (HW) equilibrium.

Differentiation between pike-perch populations. To measure the degree of genetic differentiation among the different populations, Wright's F_{ST} (θ) was estimated. The F-statistics (F_{IS} and F_{ST}) calculated according to Weir and Cockerham (1984) and their statistical significance of departure from zero were assessed by GENETIX 4.05 software (Belkhir et al. 1996) using 1000 permutations. All significance values resulting from multiple comparisons were corrected for type I errors by the Bonferroni correction method using an initial significance level of 0.05. Assignment analysis was performed using the GeneClass 1.0.02. self-assignment test (Cornuet et al. 1999), with the leave-one-out procedure and Bayesian option.

The ML-Relate software (Kalinowski et al. 2006) calculates maximum likelihood estimates of relatedness (r) and relationship. The ML estimate of r, as described by Wagner et al. (2006), may be interpreted as the proportion of genes "identical by descent" (IBD) between two individuals or groups of individuals. As for the ML relationship between a pair of individuals, it was calculated by inserting the probability that a specific genealogical relationship shares 0, 1, or 2 alleles into the likelihood equation and determines which relationship yields the highest likelihood. Average pairwise relatedness (r_{xy}) and relationship of all individuals to each other from samples (Nebhana – all other populations) was calculated at the nine microsatellite loci.

The Bayesian methodology of STRUCTURE (Version 2.0) (Pritchard et al. 2000) was implemented to determine the level of genetic substructure in the data set independently of sampling areas. To estimate the number of subpopulations (K), ten independent runs of K = 1–20 were carried out at 100 000 Markov chain Monte Carlo (MCMC) repetitions and performed with a burn-in period of 100 000 using no prior information and assuming admixture and independent allele frequencies. The most probable number of populations was taken using the log-likelihood of K. Individuals had then been assigned to each subpopulation based on allele frequency patterns. Each pre-defined population was accepted to belong to a certain cluster if the membership probability was higher than 50%. Otherwise, that population was assigned as not belonging to any group.

The net nucleotide distance was calculated between all pairs of clusters by STRUCTURE. A phylogenetic tree based on this distance was constructed using the Neighbour-Joining (NJ) algorithm with MEGA software (Version 5.10) (Tamura et al. 2011). Bootstrapping with 100 replicates of estimated allele frequencies in each cluster and the construction of a consensus tree based on Reynolds distance (Reynolds et al. 1983) were carried out using the SEQBOOT and CONSENSE modules of the PHYLIP software package (Felsenstein 1993). This consensus tree has the same topology as the NJ tree based on the net nucleotide distance. STRUCTURE outputs for each K were compiled with the software CLUMPP (Jakobsson and Rosenberg 2007) using the Greedy K algo-

rithm. CLUMPP aligns multiple replicate analyses of the same data set and creates an infile for the software DISTRUCT (Rosenberg 2004), which creates a graphical representation of the mean STRUCTURE outputs for a chosen K.

Genetic distances between populations were calculated using NEI's D_A distance (Nei et al. 1983) by GENETIX 4.05 software (Belkhir et al. 1996). A phylogenetic tree was constructed using the NJ algorithm with the NEIGHBOR and TREEVIEW modules of the PHYLIP software package (Felsenstein 1993). The NJ tree was drawn with TreeView (Version 1.6.6) (Page 2001). Bootstrapping with 100 replicates was used to test the statistical strength of the branches and the construction of a consensus tree was carried out using the CONSENSE module of the PHYLIP software package (Felsenstein 1993). For comparative reasons already published data on one year-class (2007) of wild pike-perch from Lychensee, Germany (LY7) and two Uzbek pike-perch populations (Amu-Darya River delta (AMU) and Syr-Darya River (SYR); Khurshut and Kohlmann 2009) were included in the NJ tree construction.

A study of Kohlmann et al. (2013) detected two major haplotypic lineages A and B of pike-perch based on restriction enzyme analysis of the mitochondrial cytochrome *b* gene. Haplotypes A appeared to be dominant in North-East Europe while the lineage consisting of haplotypes B might be restricted to South-East Europe. The relative proportions of these haplotypes A and B were calculated for each population based on data from Kohlmann et al. (2013) and added to the NJ tree.

RESULTS

Genetic diversity of pike-perch microsatellites and populations. The nine microsatellite loci genotyped were found to be polymorphic in all populations except loci *MSL-2* and *MSL-8* which were monomorphic in some populations (Table 2). The total number of alleles recorded over all loci was 69. The number of alleles at single loci varied from 4 (locus *MSL-7* and *MSL-8*) to 13 (locus *MSL-1*), with an average of 7.66 alleles per locus.

The allelic richness (A_r) per locus, estimated after rarefaction method, ranged from 2.11 in Sidi Salem to 4.73 in the Lake Müggelsee and seems to have the same range as A_m values (Table 2). The unbiased expected heterozygosity (H_e) varied between 0.388 ± 0.244 in the Nebhana reservoir and

0.620 ± 0.177 in the Lac d'Aydat (Table 2). Statistically significant differences between populations were observed in A_r and A_p values (ANOVA test, $P < 0.001$ for A_r and for A_p between populations), but the differences between mean H_e values were not significant (Kruskal-Wallis test, $P > 0.05$). Nevertheless, both genetic diversity estimators (A_r and H_e) showed higher values in the native, wild population. In fact, the most variable population was the German wild Lake Müggelsee population and the least variable were the two introduced Tunisian and the farmed Czech populations (Figure 1). The reduction of genetic variability of these populations is supported by a genetic bottleneck signature detected by the Wilcoxon signed rank test (Table 3). Secondly, the farmed Netherlands population also showed a weak genetic variability compared to the other European ones.

Statistically significant deviations from Hardy-Weinberg equilibrium occurred in three populations (Nebhana, the Netherlands, and Lac d'Aydat) after Bonferroni corrections. Within these populations, only the French Lac d'Aydat population showed evidence of a heterozygote deficit at loci *MSL-3* and *MSL-8* possibly due to the presence of null alleles as indicated by the MICROCHECKER software. However, after elimination of both loci F_{IS} remained significant, indicating a probable Wahlund effect.

Genetic differentiation between pike-perch populations. The evaluation of genetic differentiation between populations by F_{ST} values revealed significant differences between most pairs of populations even after Bonferroni corrections (Table 4). F_{ST} values between the two Tunisian populations were low, as could be expected, because the Sidi Salem population originated from the Nebhana population, but highly significant ($P < 0.001$). The highest levels of differentiation were observed in comparisons with the Czech farmed population, which seemed to be genetically different from the rest of populations. Moreover, the high average accuracy of the self-assignment tests (83.55% of individuals were correctly classified according to the population of their origin) reflected a high level of genetic differentiation among populations (Table 5). Remarkable misclassifications were only observed among the two introduced Tunisian and some of the French pike-perch populations.

Average pairwise relatedness values varied from 0.0035 (over Nebhana individuals – the individuals farmed in the Czech Republic) to 0.1570 (over

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Table 2. Genetic variability of pike-perch at nine microsatellite loci

Locus	SSL	NEB	NTL	MUG	CZR	CEG	ARD	EPU	LPO	LCR	LMD	ETA	RSF	RBA	RTO	LAY	RHA
MSL-1																	
n	50	54	41	50	23	31	30	27	22	37	30	30	27	30	30	16	18
H_e	0.6257	0.5647	0.7543	0.7980	0.4454	0.7197	0.7254	0.6897	0.6184	0.6920	0.7045	0.7627	0.7666	0.7198	0.5339	0.7661	0.7333
Phw	ns	*	***	ns	ns	*	ns	ns	ns	ns	*	**	ns	ns	ns	*	ns
MSL-2																	
n	50	54	41	50	23	31	30	27	22	37	30	30	27	30	30	16	19
H_e	0.0000	0.0000	0.6998	0.6988	0.6290	0.6690	0.5045	0.3529	0.3309	0.7705	0.5989	0.7299	0.5563	0.8017	0.6379	0.7500	0.5462
Phw			**	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	*
MSL-3																	
n	50	54	41	50	23	31	30	27	22	37	30	30	27	30	30	16	19
H_e	0.4655	0.5019	0.6155	0.6549	0.2638	0.6510	0.5588	0.6534	0.5761	0.5683	0.4627	0.5130	0.5388	0.6712	0.5944	0.6391	0.6330
Phw	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
MSL-4																	
n	50	54	41	50	23	31	30	27	22	37	30	30	27	30	30	16	19
H_e	0.4467	0.4422	0.1177	0.7339	0.3478	0.5653	0.6791	0.6212	0.4852	0.6231	0.6847	0.4842	0.7275	0.6718	0.6401	0.6633	0.6899
Phw	**	ns	ns	ns	ns	**	ns	**	ns	**	*	ns	ns	*	ns	ns	ns
MSL-5																	
n	50	54	41	49	23	31	30	27	22	37	30	30	27	30	30	16	19
H_e	0.4242	0.3311	0.0244	0.3063	0.3217	0.4130	0.0966	0.2397	0.4345	0.2936	0.5893	0.3452	0.3187	0.3102	0.4249	0.5242	0.4367
Phw	ns	ns	rien	ns	ns	**	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
MSL-6																	
n	50	54	41	50	23	31	30	27	22	37	30	30	27	30	30	16	19
H_e	0.6537	0.6135	0.1581	0.5574	0.4145	0.6721	0.5266	0.7491	0.6607	0.6920	0.6531	0.4938	0.5751	0.6661	0.5237	0.6593	0.6316
Phw	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
MSL-7																	
n	50	54	41	50	23	31	30	27	22	37	30	30	27	30	30	16	19
H_e	0.2982	0.3785	0.5917	0.6277	0.5101	0.2522	0.5062	0.4647	0.5106	0.1736	0.5079	0.5085	0.4885	0.4723	0.2350	0.5262	0.3087
Phw	ns	ns	***	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns
MSL-8																	
n	50	54	41	50	23	31	30	27	22	37	30	30	27	30	30	16	19
H_e	0.0000	0.0000	0.1397	0.4022	0.0000	0.6425	0.0966	0.3767	0.2125	0.5046	0.4944	0.4808	0.4088	0.2441	0.6299	0.2339	0.1024
Phw			ns	ns		ns	ns	ns	ns	ns	ns	ns	*	ns	ns	***	ns
MSL-9																	
n	50	53	41	49	23	31	30	27	22	37	30	30	27	30	30	16	19
H_e	0.6184	0.6600	0.7127	0.7174	0.7217	0.7668	0.7333	0.7296	0.6850	0.6946	0.6192	0.7130	0.7428	0.7429	0.5559	0.8226	0.7767
Phw	***	ns	***	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
A_m	2.11	2.22	3.44	5.44	2.33	4.00	3.11	4.33	3.78	3.89	3.78	4.22	4.22	3.67	3.78	4.67	3.89
A_r	2.11	2.14	3.07	4.73	2.30	3.64	3.04	4.01	3.61	3.55	3.54	3.85	3.98	3.45	3.45	4.67	3.81
Phw population	ns	***	***	ns	ns	**	ns	*	ns	ns	ns	**	*	ns	ns	***	ns

n = sample size, A_m = mean number of alleles per locus, A_r = allelic richness, H_e = expected heterozygosity, Phw = significance level of Hardy-Weinberg probability test, ns = not significant

[1]three-letter codes specify the populations location – see Table 1

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

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Table 3. Wilcoxon test for heterozygosity excess under TPM and SMM mutation models

Populations	TPM		SMM	
	He/Hd	*P*	He/Hd	*P*
Nebhana (TUN)	6/1	0.01563	6/1	0.03906
Sidi Salem (TUN)	7/0	0.00781	7/0	0.00781
Vodňany (CZR)	8/0	0.00391	6/2	0.03724

TPM = two-phase model of mutation, SMM = stepwise mutation model, He = number of loci showing heterozygosity excess, Hd = number of loci showing heterozygosity deficiency, *P* = statistical probability under the Wilcoxon test that the population exhibits overall heterozygosity excess over all loci given in the model

Nebhana individuals – Sidi Salem individuals). The maximum percentage of relationship was observed between the two Tunisian populations, followed by pairwise Tunisian-French populations and then Tunisian-German populations (Table 6).

The admixture analysis with STRUCTURE indicated that a K-value of 8 clusters provided the best fit because of its consistent Ln Prob value and the smallest standard deviation (–10218.2). The STRUCTURE analysis with optimal K = 8 (Figure 2A) revealed five relatively "pure" clusters (as characterized by a single or only few colours): the two Tunisian populations together in one cluster, the Czech and French Lac de Madine together in a second cluster, and the Netherlands, German Lake Müggelsee, and French Ardres each in a distinct cluster. The remaining clusters more or less "admixed" were characterized by the presence of more colours. The NJ tree (Figure 2B), based on the net nucleotide distances and drawn to better visualize the levels of differences between the STRUCTURE clusters, showed that the Tunisian cluster is closer related to the French

Ardres cluster forming with it a group supported by a medium-sized bootstrap value of 55%.

The D_A genetic distances displayed a large variation among pairs of populations ranging from 0.031 (between the two Tunisian populations) to 0.884 (between Nebhana and the population farmed in the Czech Republic) (Table 4). The NJ tree based on D_A genetic distances (Figure 3) supported the clustering of Tunisian populations (93% bootstrap value) within the cluster of French populations including the distinct Czech Republic farmed population. The two German wild populations (Lakes Müggelsee and Lychensee from year-class 2007) formed a sister group to the French-Tunisian-Czech group supported by a bootstrap value of 65%. The Uzbek wild populations were separated from all other populations and appeared as an outgroup with a bootstrap value of 100%. This result is in agreement with the groupings inferred from the NJ tree based on the net nucleotide distances and the evident distinction of the cluster formed by the French Lac de Madine and the Czech populations (Figure 3).
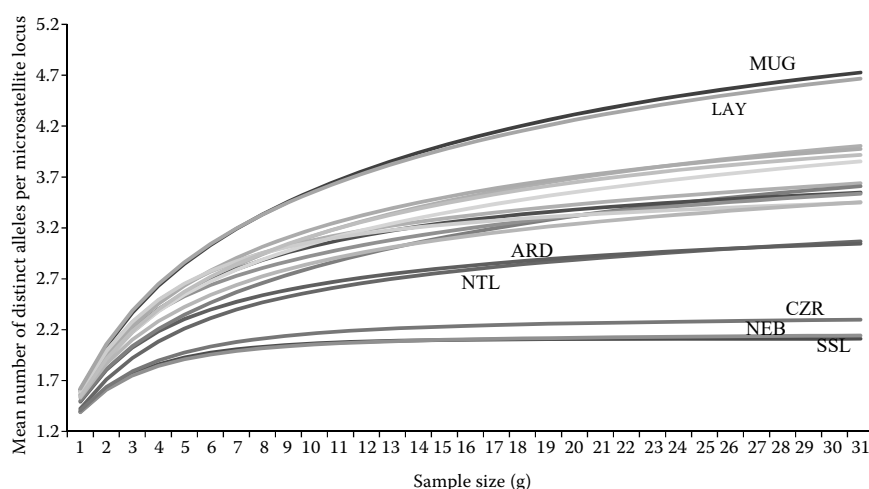

Figure 1. Mean number of distinct alleles per microsatellite locus as a function of standardized sample size obtained by ADZE program (Szpiech at al. 2008)

three-letter codes specify the samples location – see Table 1

Table 4. Matrix of F_{ST} values (above diagonal) and NEI's D_A distance (below diagonal) between pairs of populations[1] based on a data set of microsatellite loci

	SSL	NEB	NTL	MUG	CZR	CEG	ARD	EPU	LPO	LCR	LMD	ETA	RSF	RBA	RTO	LAY	RHA	LY7	AMU	SYR
SSL		0.038	0.350	0.213	0.460	0.207	0.167	0.160	0.202	0.184	0.274	0.201	0.130	0.187	0.193	0.163	0.137	0.324	0.339	0.325
NEB	0.031		0.378	0.233	0.473	0.228	0.208	0.162	0.209	0.206	0.287	0.233	0.157	0.206	0.248	0.186	0.15	0.314	0.339	0.331
NTL	0.469	0.534		0.134	0.435	0.192	0.199	0.203	0.225	0.188	0.302	0.127	0.147	0.148	0.208	0.155	0.200	0.357	0.306	0.264
MUG	0.305	0.341	0.178		0.323	0.122	0.111	0.099	0.133	0.096	0.171	0.074	0.068	0.083	0.130	0.052	0.077	0.140	0.210	0.181
CZR	0.840	0.884	0.803	0.751		0.318	0.411	0.334	0.341	0.246	0.156	0.261	0.314	0.312	0.271	0.294	0.387	0.515	0.349	0.296
CEG	0.272	0.304	0.274	0.258	0.662		0.124	0.083	0.104	0.096	0.186	0.104	0.072	0.070	0.099	0.075	0.065	0.287	0.235	0.204
ARD	0.175	0.227	0.240	0.179	0.872	0.199		0.105	0.159	0.153	0.249	0.12	0.043	0.096	0.163	0.086	0.089	0.277	0.279	0.246
EPU	0.175	0.173	0.269	0.180	0.619	0.150	0.150		0.071	0.098	0.184	0.115	0.046	0.096	0.109	0.081	0.063	0.228	0.250	0.221
LPO	0.253	0.228	0.288	0.235	0.573	0.173	0.224	0.108		0.146	0.217	0.100	0.105	0.112	0.173	0.084	0.075	0.284	0.266	0.229
LCR	0.220	0.251	0.253	0.177	0.383	0.176	0.238	0.160	0.235		0.116	0.074	0.066	0.075	0.038	0.039	0.091	0.277	0.239	0.218
LMD	0.418	0.441	0.564	0.391	0.212	0.428	0.511	0.370	0.431	0.212		0.129	0.156	0.136	0.148	0.139	0.202	0.309	0.261	0.214
ETA	0.245	0.295	0.151	0.136	0.416	0.194	0.178	0.191	0.153	0.124	0.243		0.065	0.066	0.093	0.056	0.102	0.265	0.242	0.205
RSF	0.140	0.171	0.183	0.132	0.593	0.141	0.070	0.085	0.167	0.116	0.320	0.116		0.059	0.088	**0.032**	0.042	0.251	0.225	0.202
RBA	0.231	0.257	0.191	0.165	0.620	0.141	0.147	0.170	0.185	0.134	0.280	0.122	0.115		0.104	**0.028**	0.057	0.246	0.235	0.194
RTO	0.222	0.311	0.274	0.238	0.416	0.171	0.244	0.172	0.273	0.065	0.269	0.149	0.147	0.179		0.086	0.128	0.324	0.264	0.234
LAY	0.191	0.220	0.206	0.125	0.564	0.177	0.144	0.160	0.153	0.091	0.322	0.120	0.091	0.086	0.165		**0.019**	0.235	0.193	0.168
RHA	0.144	0.154	0.259	0.143	0.831	0.125	0.130	0.107	0.117	0.154	0.430	0.173	0.086	0.110	0.210	0.068		0.232	0.228	0.205
LY7	0.414	0.385	0.539	0.187	1.486	0.522	0.396	0.318	0.414	0.457	0.591	0.419	0.388	0.393	0.570	0.364	0.320		0.326	0.295
AMU	1.068	1.006	0.979	0.855	1.686	1.086	1.093	1.028	1.058	0.935	1.389	1.002	0.903	1.059	1.104	0.861	0.882	1.144		0.060
SYR	1.292	1.291	0.843	0.821	1.420	1.088	1.077	1.056	1.003	1.016	1.220	0.918	0.965	0.939	1.138	0.894	0.938	1.142	0.324	

values in bold face are not significant after the Bonferroni correction; all the other values are significant
[1]three-letter codes specify the samples location – see Table 1

Additionally, the microsatellite based NJ tree showed only a weak association between population clusters according to these nuclear DNA markers and the distribution of the two cytochrome *b* lineages A and B as a single mitochondrial DNA marker. In particular, the introduced French populations forming a single cluster based on microsatellites displayed variable proportions of both cytochrome *b* lineages: haplotype A was fixed or dominating in most populations while haplotype B was less frequent with larger proportions only in the populations from Etang des Aulnes, Ardres, and Lac de Pont (Figure 3). Interestingly, the two Tunisian populations fixed for haplotype B were closest related to the French Retenue de Hautefage population in which haplotype A dominated.

DISCUSSION

Genetic diversity of pike-perch populations.
The numerous introductions of pike-perch expanded its geographical range. Such transplantations

can affect the native wild populations which can be mixed or introgressed with the introduced ones.

The level of genetic variability of German pike-perch as found in the wild Lake Müggelsee population is comparable to that observed by Khurshut and Kohlmann (2009) (A_m = 3.11–3.78 alleles per locus and H_e = 0.471) and Eschbach et al. (2014) (A_m = 5.7 and H_e =0.62) in wild populations from Uzbekistan and Germany, respectively, using the same microsatellite loci. Based on microsatellite loci isolated from related species, other studies have examined the genetic variability and structure of wild and introduced pike-perch populations. Indeed, the comparison with native populations (three coastal and five freshwater) from Finland (Saisa et al. 2010) showed that allelic richness and H_e were similar to the Lake Müggelsee population (A_r = 3.3–5.5 and H_e = 0.3–0.46). Likewise, Bjorklund et al. (2007) genotyped 18 pike-perch populations spread over Fennoscandia and found comparable genetic variability levels (mean A_r = 4.62 ± 0.83 in northern samples and 3.38 ± 0.62 in
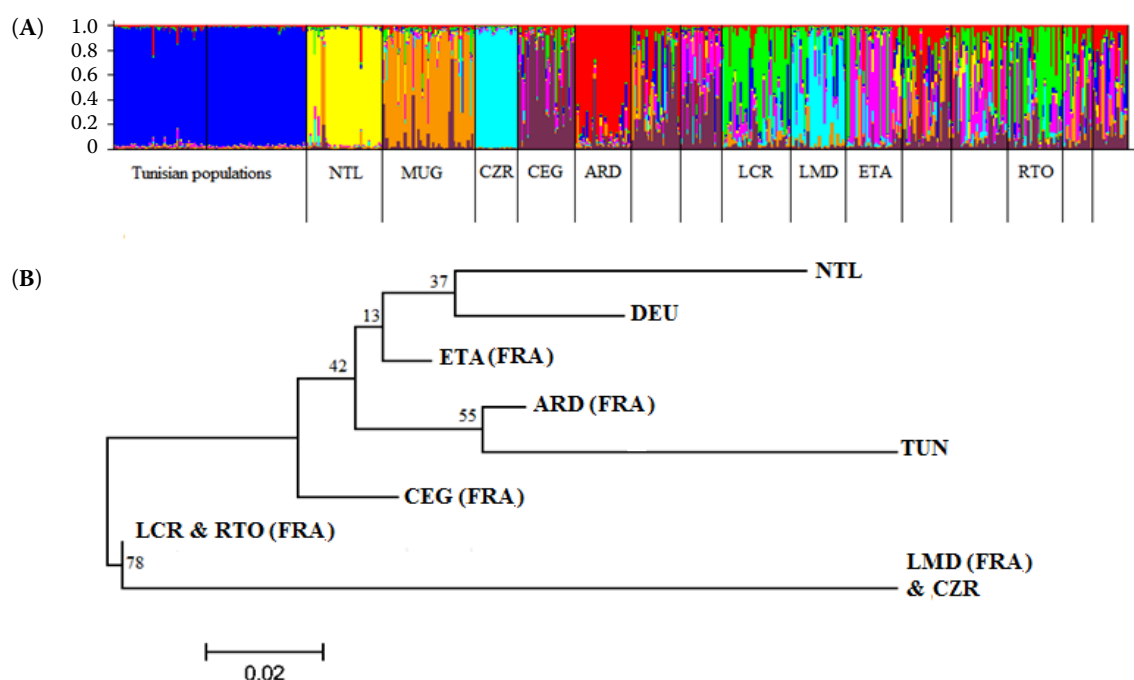
Figure 2. Results of the structure analysis with optimal K = 8 (**A**), Neighbour-Joining tree based on the net nucleotide distances showing the relationships between the eight clusters (**B**)

three-letter codes specify the samples location – see Table 1

southern samples; mean gene diversities = 0.55 ± 0.11 in northern samples and 0.49 ± 0.097 in southern samples) as observed in the studied German wild population.

In contrast, lower levels of genetic diversity have been observed in one farmed (the Czech Republic) and two introduced populations (Tunisia) in the present study compared to the wild ones mentioned above. The decrease of allelic diversity in domesticated stocks and the large-scale loss of the original genetic diversity within the species (loss of alleles at loci *MSL-2* and *MSL-8*) could have been the result of the stocking program if low numbers of individuals were introduced. As a consequence, high inbreeding levels and genetic drift may occur. In fact, these three populations revealed a genetic bottleneck signature detected by the Wilcoxon signed rank test. Also, the Netherlands population showed a weaker genetic variability compared to the French ones due to the fact that it is a farmed population. In addition to the usually low number of breeders used in hatcheries, the type of controlled reproduction (fully artificial by hormonal stimulation and stripping of eggs and sperm or semi-artificial by providing spawning substrates in the rearing tanks or ponds) might also influence the genetic variability of farmed pike-perch stocks.

However, the effect of low numbers of spawners on the genetic bottleneck is expected to be much more important. Different authors have stressed the reduced genetic variability of farmed stocks in other fish species as well such as the Atlantic salmon (*Salmo salar*), where a loss of genetic variability at the hatchery was detected resulting from a random drift due to a small number of founders (Verspoor 1988), the common carp (*Cyprinus carpio*), in which domestication caused a reduction of nuclear (microsatellite) and mitochondrial DNA variability (Kohlmann et al. 2005; Kohlmann and Kersten 2013), and the sea bass (*Dicentrarchus labrax*), where the sample from the gulf of Tunis showed a low level of genetic variability probably owing to its farmed origin (Bahri-Sfar et al. 2005).

In contrast, Poulet et al. (2009) characterized the genetic diversity of an introduced pike-perch population in the Rhône delta (France) and highlighted a high genetic diversity compared to 18 native populations from the Baltic Sea drainage genotyped with the same microsatellite loci as in Bjorklund et al. (2007) (A_{Native} = 4.2 ± 1.3, A_{Intro} = 4.9 ± 0.9 and H_{Native} = 0.5 ± 0.1, H_{Intro} = 0.7 ± 0.04). This high genetic diversity observed in French populations might be explained by multiple introduction events (Poulet et al. 2009). Indeed, the Lac

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Table 5. Results of the pike-perch self-assignment test

Original population	SSL	NEB	NTL	MUG	CZR	CEG	ARD	EPU	LPO	LCR	LMD	ETA	RSF	RBA	RTO	LAY	RHA	Correct assignment (%)
SSL	36	14	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	72.0
NEB	13	41	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	75.9
NTL	–	–	39	2	–	–	–	–	–	–	–	–	–	–	–	–	–	95.1
MUG	–	–	–	44	–	–	1	–	1	–	–	–	2	–	–	–	2	88.0
CZR	–	–	–	–	23	–	–	–	–	–	–	–	–	–	–	–	–	100.0
CEG	–	–	–	–	–	26	1	–	1	–	–	–	–	1	1	–	1	83.9
ARD	–	–	–	–	–	–	29	–	–	–	–	–	1	–	–	–	–	96.7
EPU	–	–	–	–	–	–	1	23	1	–	–	–	2	–	–	–	–	85.2
LPO	–	–	–	–	–	–	–	–	18	–	–	1	1	–	–	–	2	81.8
LCR	–	–	–	–	–	–	–	–	–	33	1	1	–	1	–	–	1	89.2
LMD	–	–	–	–	–	–	–	–	–	1	29	–	–	–	–	–	–	96.7
ETA	–	–	–	–	–	–	–	–	2	3	–	23	–	1	1	–	–	76.7
RSF	–	–	–	–	–	1	3	2	1	1	1	–	18	–	–	–	–	66.7
RBA	–	–	–	–	–	1	–	–	–	1	–	–	1	23	1	1	2	76.7
RTO	–	–	–	–	–	1	–	2	–	1	–	–	1	–	25	–	–	83.3
LAY	–	–	–	–	–	1	–	–	–	–	–	–	1	1	–	10	3	62.5
RHA	–	–	–	–	–	–	–	–	1	–	–	1	–	–	–	–	17	89.5

[1]three-letter codes specify the samples location – see Table 1

d'Aydat population showed a genetic variability comparable to that in the German wild population and a relatively large distinctness (A_p = 0.67). Pike-perch is a very popular game fish in France, and a tremendous number of introductions occurred in French drainages increasing the number of individuals introduced and the probability that these fish have distinct genetic origins, what finally leads to an increased gene pool of these populations (Poulet et al. 2009).

Heterozygote deficit (F_{IS} < 0) has been detected in the Lac d'Aydat population; the most evident explanation for this is the potential impact of population substructure "Wahlund effect" on populations. However, Nebhana and the Netherlands populations showed heterozygote excess (F_{IS} > 0). This finding might be explained by the associations of alleles that have a selective heterozygote advantage.

Genetic differentiation between pike-perch populations. The high differentiation level observed between the pike-perch populations and the high average accuracy of the self-assignment tests may be related to genetic drift. In fact, drift has been an important factor determining the genetic diversity in the Tunisian, Czech, and the Netherlands populations and the long-term effective population sizes have been small with a low number of founders as indicated by the positive bottleneck signal for some of these populations. After introduction, already existing differences could have increased due to genetic drift and/or selection. It could differentiate populations from each other when gene flow was absent, ceased or became negligible. Indeed, random factors within each population contribute to their later genetic separation by genetic drift (Bjorklund

Table 6. Pairwise mean relatedness (r_{xy}) and percentage of relationship between the Nebhana and all other studied pike-perch populations

Population	Nebhana	
	r_{xy}	(%)
SidiSalem (TUN)	0.157	30.778
Venlo (NTL)	0.0060	0.090
Lake Müggelsee (DEU)	0.0106	1.667
Vodňany (CZR)	0.0035	0.000
Lac de Pont (FRA)	0.0341	5.135
Etang du Puits (FRA)	0.0265	3.909
Complexe d'Eguzon (FRA)	0.0198	2.927
Ardres (FRA)	0.0248	3.580
Lac de Crescent (FRA)	0.0281	4.855
Lac de Madine (FRA)	0.0186	1.235
Etang des Aulnes (FRA)	0.0128	1.543
Retenue de Saint-Ferréol (FRA)	0.0229	3.978
Retenue de Bage (FRA)	0.0179	2.099
Retenue du Tordre (FRA)	0.0179	2.593
Lac d'Aydat (FRA)	0.0246	4.398
Retenue de Hautefage (FRA)	0.0454	7.310

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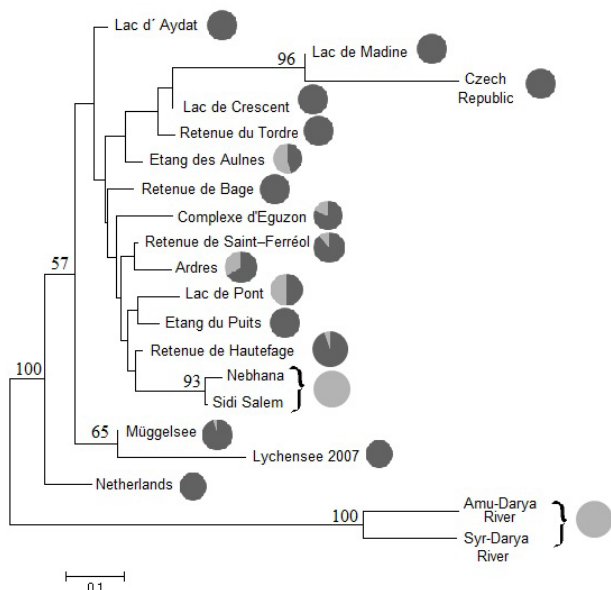
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Figure 3. Neighbour-Joining tree of pike-perch populations based on 9 microsatellite loci and NEI's D_A distance. Relative proportions of haplotypes A (black colour) and B (grey colour) were also calculated based on data of Kohlmann et al. (2013) and attributed to the studied populations

et al. 2007). Thus, the STRUCTURE software splits those populations as divergent groups with a few fairly admixed individuals.

Some pairwise comparisons of French populations highlighted non-significant F_{ST} values, what can be explained by gene flow through introductions probably because anglers associations were supplied from the same fish farm and by the history of the introduced progenitors, taking into account the usually very small number of source populations and the limited number of breeders (at least at the beginning of introductions).

In contrast, the significant genetic differentiation among the other French populations may stem from repeated introductions of pike-perch from different fish farms and from differences in population dynamics due to selection effects. In the study of Saisa et al. (2010) some of the loci showed especially high divergence between populations from different environments possibly indicating relation to adaptively important genes (the samples of northernmost lake populations differed strongly from the southern lake populations).

In the present study, the genetic structure of introduced pike-perch populations in Tunisia was characterized, and we tried to trace their origin. The average pairwise relatedness values highlight

a closer relationship between Tunisian and French populations than between Tunisian and German ones. In fact, most other introduced freshwater fish species were imported in the same year from French hatcheries, e.g. common carp, roach (*Rutilus rubilio*), and rudd (*Scardinius erythrophthalmus*). This is supported by the STRUCTURE results: the two Tunisian populations cluster with the French population of Ardres, whereas the Netherlands and the German Lake Müggelsee population form a distinct cluster. The remaining admixed populations testify, on the one hand, that the pike-perch breeders used in stocking programs would be genetically close and, on the other hand, that a re-stocking and transfer program within sampling localities may produce an increasing gene flow.

Tunisian populations may share the same lineage as French pike-perch which is admixed, as highlighted by the clustering observed in the NJ trees based on the net nucleotide distances and on D_A genetic distances. This is also sustained by the relative proportions of cytochrome *b* haplotypes A and B in the studied populations. Only haplotype B was detected in Tunisian samples. In contrast, haplotype A was the most frequent type found in all French populations and haplotype B is present there with an average proportion of only 14.36%. This discrepancy might be due to different source populations originally introduced into France, multiple introductions, founder effects, genetic drift, and natural selection or combinations of any of these factors. A natural contact zone of both lineages exists in Germany and the Czech Republic (Kohlmann et al. 2013; Eschbach et al. 2014).

CONCLUSION

In conclusion, the present results demonstrate that despite the genetic differences, the studied populations still cluster or differentiate according to their phylogeographic origin. The Tunisian populations seem to be introduced from a French hatchery where the brood stock has the haplotype B of the mitochondrial cytochrome *b* gene.

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